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Journal OF THE Fisheries Research Board OF Canada

Laboratory Studies of the Accommodation of Some Crude and Residual Fuel Oils in Sea Water[1]

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GORDON, D. C. JR., P. D. KEIZER, AND N. J. PROUSE. 1973. Laboratory studies of the accommodation of some crude and residual fuel oils in sea water. J. Fish. Res. Board Can. 30: 1611–1618.

Concentrations of oil accommodated in sea water under laboratory conditions are directly related to the amount added and the degree of turbulence, but inversely related to temperature. The major fraction (87–98%) of this oil is in particulate form ranging in size from about 1 to 30 μ, presumably small droplets. The exact ratio of the particulate to subparticulate fractions is directly related to the apparent viscosity of each individual oil. Of the total amount of oil initially added, 9–15% appeared in sea water at the end of the 7-days experiments. Except in the proximity of recent oil slicks, the oil concentrations observed in these experiments are one to two orders of magnitude greater than the concentrations generally observed in the marine environment off eastern Canada.

GORDON, D. C. JR., P. D. KEIZER, AND N. J. PROUSE. 1973. Laboratory studies of the accommodation of some crude and residual fuel oils in sea water. J. Fish. Res. Board Can. 30: 1611–1618.

Les concentrations de pétrole que peut accomoder l'eau de mer sont en fonction directe des quantités qui y sont versées et du degré de turbulence, mais en fonction inverse de la température. La plus grande partie (87–98%) de ce pétrole est sous forme particulaire, de taille variant d'environ 1 à 30 μ, présumément de petites gouttelettes. Le rapport exact de la fraction particulaire à la fraction subparticulaire est directement lié à la viscosité de chaque pétrole individuel. Sur la quantité totale de pétrole ajouté au début, 9–15% est apparu dans l'eau de mer à la fin de l'expérience de 7 jours. Sauf dans le voisinage des nappes récentes, les concentrations de pétrole observées au cours de ces expériences sont d'un à deux ordres de grandeur plus fortes que les concentrations généralement observées dans le milieu marin de la côte est du Canada.

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[1]Bedford Institute of Oceanography Contribution.

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PETROLEUM hydrocarbons are soluble to a limited degree in water (McAuliffe 1969) and can form fine dispersions (Parker et al. 1971) and discrete particles (Forrester 1971) in the water column. It is therefore reasonable to expect that a measurable portion of any oil spilled into the sea will enter the water column where it has greater chances of coming into contact with marine organisms, for example zooplankton (Conover 1971). Anv effects on the marine biota will depend upon the quantity, physical form, and chemical composition of oil entering sea water. With the exception of the work of Parker et al. (1971), Boylan and Tripp (1971), and Smith and MacIntyre (1971), little research has been directed to this topic. To understand more fully the potential availability of petroleum hydrocarbons to marine organisms inhabiting the water column (both in the natural environment and in experimental situations devised to test the effects of hydrocarbons), we undertook a series of laboratory experiments to study the accommodation of three different types of oil in sea water under different experiment conditions. These experiments also provided some information on the quantitative fate of petroleum hydrocarbons added to but not appearing in sea water.

Methods

Experiments were conducted in 4-liter glass separatory funnels suspended in an indoor sea water tank. Three liters of Bedford Basin surface water were placed in each funnel after screening through 160-μ plankton netting to remove large organisms and detrital particles. Oil solutions were prepared by dissolving 100 mg of oil (Venezuelan crude, No. 2 fuel, and No. 6 fuel) in 10 ml of *n*-hexane (the asphaltene fraction did not dissolve completely so solutions were shaken vigorously just before subsamples were drawn). A known volume of these solutions was added by syringe to the appropriate funnels (not controls). The oil immediately formed a visible film on the water surface. The tops of the funnels protruded 10 cm above the tank and an electric fan set a few meters away generated a breeze of about 6 knots over all funnels. Experimental temperature was controlled by regulating the temperature of water in the tank. Incandescent lights over the tank (about 100 lux) were on for 12 hr daily.

The design of individual experiments is summarized in Table 1. Funnel stoppers were removed except for

TABLE 1. Design of experiments.

Expt. no.	No. funnels	Duration (*hr*)	Temp. (C)	Oil studied	Concn added (*mg/liter*)	Shaking[a] (*sec twice daily*)	Funnel condition[b]	Analyses performed[c]	Sampling frequency
1	3	336	21	No. 2	10	30	O	1a	Daily
2	4	336	21	crude	3.8	30	O	1a	Daily
3	12	168	8	No. 2,No. 6, crude	5	none, 30, 60	O	1b–6	End of experiment
4	12	168	8	No. 2,No. 6 crude	1,5,10	30	O	1b–6	End of experiment
5	8	168	21	No. 2,No. 6 crude	5	30	O	1b–6	End of experiment
6	8	168	2	No. 2,No. 6 crude	5	30	O	1b–6	End of experiment
7	4	168	8	No. 2,No. 6 crude	5	30	O	2–7	End of experiment[d]
8	13	168	19	No. 2,No. 6, crude	5	30	O,C,P,D	1b–7	End of experiment[d]
9	13	168	1	No. 2, No. 6, crude	5	30	O,C,P,D	1b–7	End of experiment[d]
10	2	168	4	crude	5	30	O,C	8	End of experiment
11	2	168	19	crude	5	30	O,C	8	End of experiment
12	3	1	21	crude	5	30[e]	C	8	End of experiment

[a]At least 17 hr before samples drawn.
[b]Funnel conditions: O, open; C, closed; P, poisoned; and D, darkened.
[c]Analyses: 1 — total oil by fluorescence spectroscopy (a — total water, b — rinse only); 2 — subparticulate oil by fluorescence spectroscopy; 3 — particulate oil by elemental analysis; 4 — particle size distribution; 5 — *p*H; 6 — dissolved oxygen; 7 — bacteria counts; and 8 — gas–liquid chromatography.
[d]Except for bacteria samples which were collected daily.
[e]Shaken just once at beginning of experiment.

certain funnels in Experiments 8–12 and all funnels when shaken. With the exception of bacteria counts, all samples were drawn from the bottom valve after separatory funnels had been removed from the tank. After the first 100–250 ml of sea water was discarded, 100 ml was collected for oil analysis. Total and subparticulate oil was determined by fluorescence spectroscopy and particulate oil by elemental analysis (Keizer and Gordon 1973). About 500 ml was drawn for subsequent (within 6 hr) determination of particle size distribution by a Model T Coulter counter, using 50 and 180 μ tubes (actual volume analyzed averaged 20 ml). The *p*H was determined by a portable Radiometer *p*H meter (Model 29) and dissolved oxygen by a Radiometer acid–base analyzer (Model PHM 71) equipped with a PO_2 module (Model PHA 930). Seawater samples for bacteria counts were collected at a depth of 5 cm below the water surface with a sterile pipette inserted through the top opening of the separatory funnels. Total viable bacteria concentrations were determined by standard plate counts made on trypticase soy agar (BBL) containing 3% NaCl after 7 days incubation at 20 C.

In the last three experiments, 2 liters of sea water were drawn for analysis of *n*-alkanes and isoprenoids by gas–liquid chromatography (GLC). In Experiment 12, samples were immediately filtered through 1.2 μ Millipore® filters (which had been previously extracted with methylene chloride) and the filters were placed in glass bottles containing 40 ml of methylene chloride and soaked overnight. The filtrates and the whole water samples from Experiments 10–11 were extracted three times with 40 ml of methylene chloride. All extracts were concentrated and the solvent changed to *n*-hexane in a Kudurna–Danish evaporator. The *n*-hexane concentrate, approximately 500 μliters, was eluted through a silica gel and alumina column (5:1 w/w) with redistilled *n*-hexane and the first fraction (containing mostly paraffins) was collected and concentrated to 500 μliters for GLC analysis. Samples were stored in glass vials with septum caps and analyzed as soon as possible using a Perkin-Elmer 990 gas chromatograph (equipped with a 300 ft by 0.02 inch ID Apiezon L wall coated open tubular column and flame ionization detector; injector temp. 270 C, detector temp. 250 C; column kept at 70 C for 2 min after injection and then programed at 4 C/min to 220 C or two 6 ft by 0.125 inch OD columns packed with 1% SE-30 on 60/80 mesh Chromosorb W(AW) and programed from 90 to 250 C at 8 C/min. Normal hydrocarbons were identified using internal standards, and pristane and phytane using a chromatogram published by Sever and Haug (1971).

In Experiments 8 and 9, three funnels were stoppered during the entire experiment to prevent evaporation (closed), three were injected with 100 mg of potassium cyanide to retard bacterial growth (poisoned), and three were covered with several layers of heavy duty aluminum foil (except over the stopper opening) to block out most light (darkened). At the end of these experiments, after the usual samples had been drawn, the remaining water in the funnels was discarded and the inside of each funnel was rinsed twice with 20 ml of *n*-hexane to recover oil adhering to the inner surface (surface film). The total oil content of this solution was determined by fluorescence spectroscopy.

The total amount of oil present in the water (approximately 3 liters) of each funnel at the end of Experiments 8 and 9 was estimated by multiplying the sum of the particulate and subparticulate oil concentrations observed in the 100 ml sea water sample analyzed by 30. The total amount of oil remaining in each funnel was estimated by adding the amount observed in the surface film to that observed in the water. Losses of oil by evaporation, bacterial decomposition, and photochemical oxidation during the 7-day experimental period were estimated by subtracting the amounts of oil lost (oil initially added minus oil recovered) from the stoppered, poisoned, and darkened funnels, respectively, from the amount of oil lost from the corresponding open funnels (which were also unpoisoned and illuminated).

Since Bedford Basin water is chronically polluted with oil (averaging about 20 μg/liter; Keizer and Gordon 1973) it was necessary to run controls in all experiments; all concentrations reported have been corrected accordingly.

Particulate and subparticulate oil are defined as those fractions which are respectively retained or passed through a silver filter (Selas Flotronics Inc., Spring House, Pa.) of 0.8 μ porosity using a vacuum of 15 psi. This distinction, although practical, is quite arbitrary and heavily dependent upon filter composition and porosity and filtering conditions.

All solvents used were either spectrophotometric grade or redistilled (in glass) ACS grade.

Results

In the time series experiments (Fig. 1), the highest concentrations of total oil occurred within the first few hours, thereafter they dropped steadily (pre-

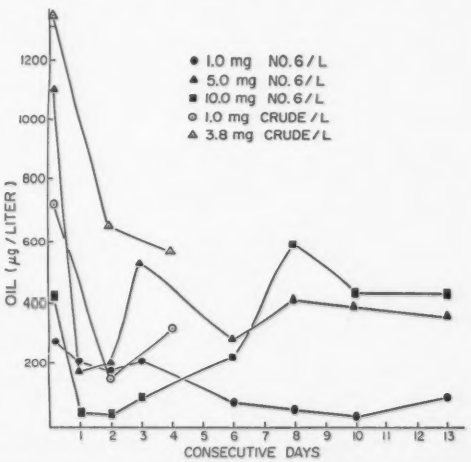
● 1.0 mg NO. 6 / L
▲ 5.0 mg NO. 6 / L
■ 10.0 mg NO. 6 / L
⊙ 1.0 mg CRUDE / L
△ 3.8 mg CRUDE / L
OIL (µg / LITER)
1200
1000
800
600
400
200
1 2 3 4 5 6 7 8 9 10 11 12 13
CONSECUTIVE DAYS

FIG. 1. Changes of total oil concentrations with time in Experiments 1 and 2.

sumably due to evaporation and bacterial decomposition) until the second or third day beyond which they fluctuated randomly. The level of concentrations observed was generally related to the concentration of oil initially added (further discussed below). On the basis of these results and for the sake of convenience, subsequent experiments (except No. 12) lasted 7 days.

Turbulence (induced by shaking) had a pronounced effect on the observed concentrations of both particulate and subparticulate oil (Fig. 2); the greater the turbulence the greater the concentrations. Low but measurable concentrations of oil were observed in the sea water of unshaken funnels, presumably due to diffusion or perhaps thermal convection. Preliminary experiments indicated that if funnels are shaken just before samples are drawn, instead of 17 hr previously as done in all experiments reported here, oil concentrations approximately double. It thus appears that a substantial amount of the oil entering sea water under turbulent conditions will return to the surface film within a few hours after the turbulence is removed, presumably in accordance to Stoke's Law.

Concentrations of particulate and subparticulate oil were generally related to the amount of oil initially added (Fig. 3). Concentrations presumably would have continued to increase if oil additions greater than 10 mg/liter had been tested. Temperature also had a measurable effect on the concentrations of particulate and subparticulate oil (Fig. 4). Concentrations of both increased by a factor of two as temperature dropped from 19–21 C to 1–2 C.

The three oils studied produced similar amounts of total oil in sea water, but the ratio of particulate to subparticulate oil varied considerably (Table 2). The No. 6 fuel oil produced the greatest particulate oil concentrations while the Venezuelan crude oil produced the greatest subparticulate oil concentrations. These differences are related to apparent viscosity and presumably chemical composition of the oils.

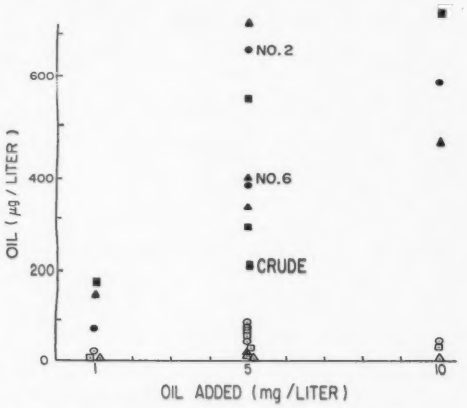

FIG. 3. The effect of the amount of oil added on the concentrations of particulate (solid symbols) and subparticulate (open symbols) oil appearing in sea water at 8 C. All funnels were shaken for 30 sec twice daily.

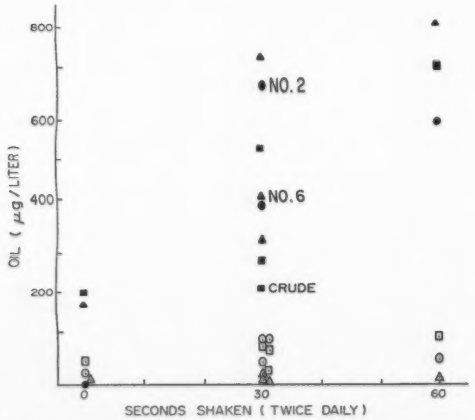

FIG. 2. The effect of turbulence on the concentration of particulate (solid symbols) and subparticulate (open symbols) oil appearing in sea water at 8 C. An initial concentration of 5 mg oil/liter was added to each funnel.

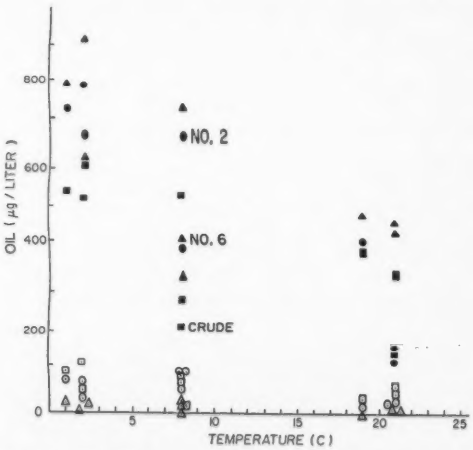

FIG. 4. The effect of temperature on the concentration of particulate (solid symbols) and subparticulate (open sumbols) oil appearing in seawater. An initial concentration of 5 mg oil/liter was added to each funnel. Funnels were shaken for 30 sec twice daily.

TABLE 2. Relative concentrations (μg/liter) of particulate, subparticulate, and total oil observed in sea water for each of the three oils studied. The concentrations were determined by averaging all data in Fig. 2–4 according to oil type and therefore have no absolute meaning.

Oil	Particulate	Subparticulate	Total[a]	Particulate/Subparticulate
Crude	435	58	493	7.5
No. 2	431	44	475	9.8
No. 6	530	13	543	40.7

[a]Particulate plus subparticulate.

Analysis by GLC indicated that *n*-alkanes and isoprenoids less than C15 were lost from sea water during 1 week at 4 C (Table 3). At 19 C (Experiment 11), all *n*-alkanes and isoprenoids less than C25 were lost.

This greater loss is presumably due to increased rates of evaporation and biodegradation at the higher temperature. Using the pristane concentration observed in Table 3 and the percent composition of pristane in the crude oil added, a total oil concentration of 640 μg/liter of sea water is estimated, in good agreement with the total oil concentrations observed using fluorescence spectroscopy and elemental analysis (Fig. 4) to analyze water from experiments conducted under the same conditions, especially temperature (4 C).

TABLE 3. GLC analysis of aliphatic hydrocarbons observed in unfiltered sea water in Experiment 10. Experiment conducted at 4 C. Crude oil only.

Compound	μg/liter	% of initial crude
C12 (*n*-dodecane)	ND[a]	0.46
C13 (*n*-tridecane)	ND	0.47
C14 (*n*-tetradecane)	ND	0.48
C15 (*n*-pentadecane)	ND	0.47
C16 (*n*-hexadecane)	1.6	0.45
C17 (*n*-heptadecane)	1.9	0.37
Pristane	2.0	0.32

[a]Not detectable.

Because of the rapid and extensive evaporation of *n*-alkanes and isoprenoids, their partitioning between the particulate and subparticulate fractions was determined in an experiment conducted in closed funnels which lasted only 1 hr (Table 4). Except for nonane (C9) and decane (C10), and probably the smaller *n*-alkanes as well, the major fraction (90–95%) of these compounds entering sea water was in a particulate form, similar to the partitioning observed using elemental analysis and fluorescence spectroscopy to estimate total oil constituents (Table 2).

The particle size distribution of particulate oil can be estimated by comparing those of controls and oil-contaminated sea water (Fig. 5). The concentration of particulate oil decreases with increasing size and approaches zero at about 20–30 μ (the volume of sample analyzed, 20 ml, determines the range of particle size that can be detected; Sheldon et al. 1972). The phytoplankton which produce the 20-μ peak in the control are apparently scavenged by oil droplets and concentrated in the surface film, hence they are not visible in oil-contaminated water.

Values of dissolved oxygen and *p*H remained at normal levels (89–94% saturation and 7.5–

TABLE 4. The partioning of three representative aliphatic hydrocarbons between particulate and subparticulate fractions in Experiment 12. The results of two replicate analyses are presented. Concentrations are μg/liter. Crude oil only.

Component	Particulate	Subparticulate[a]	Particulate/Subparticulate
C12 (*n*-dodecane)	0.8, 0.9	0.1	8,9
C16 (*n*-hexadecane)	1.2, 1.3	0.1	12,13
Pristane	0.9, 1.2	0.1	9,12

[a]Barely detectable, 0.1 μg/liter was limit of detection.

8.1, respectively) in the oil-contaminated sea water during the experiments tested, even in closed and poisoned funnels.

In a crude attempt to account for the oil not appearing in the sea water, oil budgets were calculated from the data of Experiments 8 and 9 (Table 5). At 1 C, 52% of the oil added to funnels remained in the surface film while 15% was observed in the water. Loss by evaporation, bacterial decomposition, and photochemical decomposition appeared negligible. The fate of oil at 19 C was quite different. Again the largest amount remained in the surface film, but just 29%. Only 9% was observed in the water, while 25% evaporated and 12% was degraded by bacteria. The loss by photochemical oxidation was again negligible. About 70% of the oil initially added can be accounted for when all budget terms are summed at both temperatures.

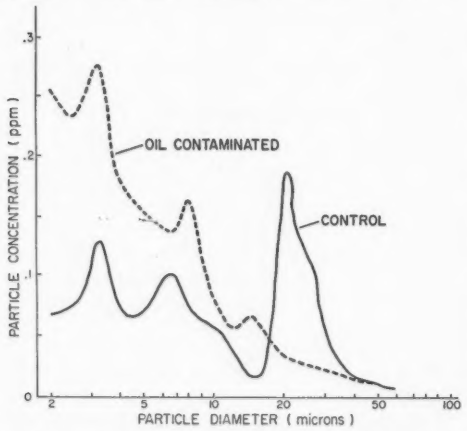

FIG. 5. Particle size spectra observed in Experiment 9. The oil-contaminated spectrum represented the average of spectra of the three different oils investigated.

Discussion

Most of the oil appearing in sea water in these experiments was particulate in form. The total concentration of oil particles larger than 2 μ detected by the Coulter counter in Experiment 9 (Fig. 5) was 439 μg/liter, while the average concentration of particulate oil detected by elemental analysis on silver filters (which have an effective median pore size slightly greater than 1 μ; Sheldon 1972) in the same experiment was 685 μg/liter. It therefore appears that about two-thirds of the oil retained on silver filters is composed of particles larger than 2 μ. The remaining one-third of the particulate oil must consist of particles between 1 and 2 μ and perhaps of some particles smaller than 1 μ which are retained due to overloading the filters, as described by Sheldon (1972). A portion of this difference could be an artifact resulting from differences in sea water volume analyzed (20 ml for Coulter counter, 100 ml for elemental analysis). The exact nature of these oil particles is not known, but it is suspected that they are small droplets of oil, as suggested by Parker et al. (1971).

TABLE 5. Oil budgets for Experiments 8 and 9. Since considerable errors are involved in estimating the various fates and few replicates were run, the results obtained using the three different oils are averaged together to estimate the behavior of a "heavy" oil. All data obtained by fluorescence spectroscopy and elemental analysis.

	Temperature			
	1 C		19 C	
Fate	μg oil	% added	μg oil	% added
1. Dissolved and suspended in sea water	2225	15	1333	9
2. Remaining in surface film	7766	52	4284	29
Total remaining	9991	67	5617	38
3. Loss by evaporation	0	0	3733	25
4. Loss by bacterial decomposition	316	2	1852	12
5. Loss by photochemical oxidation	0	0	0	0
Total loss	316	2	5585	37
Total accountable	10307	69	11202	75

Depending upon oil type, only 2–13% of the oil entering sea water in these experiments passed through a silver filter (subparticulate, Tables 2 and 4), averaging 23 μg/liter at 19–21 C (Fig. 4). This fraction consists of hydrocarbons in true solution and/or in colloidal form or perhaps particles less than 1 μ in diameter. The hydrocarbons most likely present in this fraction are those with the highest solubilities in water, for example acetylenes, aromatics, and diolefins (McAuliffe 1969). The low concentrations of subparticulate oil observed in these experiments, which are estimates based on analysis by fluorescence spectroscopy, do not appear to represent saturation levels for the reported solubilities of the above compounds are in the milligrams per liter range.

The concentrations of total oil observed in these experiments at 19–21 C are substantially lower than those few reported in the literature from similar laboratory experiments (Table 6). The differences are undoubtedly due to variations in experimental procedures, specifically the kind of oil used, the type and degree of agitation, the concentration of oil initially added, and the duration of the experiment. Most of the oil observed in the other reports was presumably also in a particulate form, as suspected by both Parker et al. (1971) and Boylan and Tripp (1971).

The results of these laboratory experiments can be used to estimate the highest concentrations of oil that can appear in a water column contaminated with oil under different environmental conditions. On January 10, 1973, a small spill of crude oil (1000 bl) occurred at the Texaco refinery at Eastern Passage, N.S. The water temperature was 0 C. Immediately under the resulting slick (0.25 m) 2 days after the spill, total oil concentrations (measured by fluorescence spectroscopy) approaching 800 μg/liter were observed, in excellent agreement with what would be predicted from the data in Fig. 4. Practically all of this oil was retained on a 1.2-μ Millipore® filter, again in close agreement with laboratory observations (Tables 2 and 4).

The chronic levels of oil pollution in sea water in various coastal inlets along the Atlantic coast of Canada and along an oceanic section between Halifax and Bermuda are much lower than the concentrations observed in these experiments, the former ranging from about 2 to 50 μg/liter (Keizer and Gordon 1973). Therefore it appears that, except for extreme cases such as under oil slicks, the capacity of sea water to accommodate oil is far from being reached, in some cases by as much as two orders of magnitude. Peake and Hodgson (1966) came to a similar conclusion when studying normal alkanes.

The unaccountable oil (about 30%) in the budget calculations represents various errors. Loss by evaporation is undoubtedly underestimated, especially at 1 C, because GLC analyses at 4 C (Table 3) indicate that aliphatic hydrocarbons (not detected by fluorescence spectroscopy) up to *n*-pentadecane (C15) are completely lost from the water. The magnitude of bacterial decomposition is also probably underestimated. Daily counts revealed that bacteria continued to grow in poisoned funnels at both temperatures and some of these could have been hydrocarbon oxidizers. There is also a chance that some of the surface film oil was removed with the sea water when the funnels were drained before rinsing with *n*-hexane. This would lead to an underestimation of oil remaining in the surface film. Despite these errors, the budget calculations give a fairly accurate picture of the relative importance

TABLE 6. Total oil concentrations observed in sea water by various workers in laboratory experiments.

Reference	Duration of experiment (*days*)	Temp. (C)	Oil(s)	Oil concentration added (*mg/liter*)	Oil concentration observed (*μg/liter*)
1. Boylan and Tripp (1971)	0.5	23	Kuwait crude	17×10^3	1453[a]
2. Smith and MacIntyre (1971)	2	25	3 fuel oils	3×10^3	860[b]
3. Parker et al. (1971)	36	Not given	Kuwait crude	50×10^3	500
	65				400
4. This paper	7	19–21	Venezuelan crude and two fuel oils	5	267

[a]Aromatic species only.
[b]Average of three oils.

of different fates at the two temperatures under the experimental conditions employed.

The lack of measurable losses by photochemical oxidation (Table 5) is not surprising. Most photochemical oxidation is caused by ultraviolet radiation, little of which is emitted by the incandescent lights over the funnels.

The budget calculations (Table 5) can be used to compare in a semi-quantitative manner the relative magnitude of the different potential fates of oil spilled on sea water in cold and warmwater environments. In cold water, the rates of evaporation and bacterial decomposition will be much lower than in warm water. Also, at any given time, cold water can accommodate greater concentrations of oil. These two factors combined suggest that other factors being equal, marine organisms living in cold water environments (i.e. all Canadian waters for at least part of the year) are much more likely to be affected by oil pollution.

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Factors Affecting the Behavior of Five Chlorinated Hydrocarbons in Two Natural Waters and their Sediments

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Water samples from the Fraser River and Georgia Strait, British Columbia were treated with different chlorinated hydrocarbons and incubated for up to 12 weeks at 13 C. 1) In the presence of bottom sediments from the same locations as the waters, no residues were found to escape into the atmosphere. 2) With the exception of lindane in ocean water, all detectable residues had moved into the sediments after 6 weeks. 3) Most of the lindane was metabolized. 4). Sterilization of the waters and sediments prevented the metabolism of lindane but had little effect on DDT and DDD. 5) Agitation of water samples containing γ-chlordane, incubated without sediment, had no effect on its disappearance, but the presence of 0.01% of a nonionic surfactant retarded this almost completely.

Oloffs, P. C., L. J. Albright, S. Y. Szeto, and J. Lau. 1973. Factors affecting the behavior of five chlorinated hydrocarbons in two natural waters and their sediments. J. Fish. Res. Board Can. 30: 1619–1623.

Des échantillons d'eau du fleuve Fraser et du détroit de Georgie, Colombie-Britannique, ont été traités avec divers hydrocarbures chlorés pendant des périodes allant jusqu'à 12 semaines à 13 C. 1) En présence de sédiments de fond provenant des mêmes endroits que l'eau, aucun résidu ne s'est échappé dans l'atmosphère. 2) A l'exception du lindane dans l'eau de l'océan, tous les résidus décelables se sont introduits dans le sédiment après 6 semaines. 3) La plus grande partie du lindane s'est transformée par métabolisme. 4) La stérilisation de l'eau et des sédiments empêche la transformation par métabolisme du lindane mais a peu d'effet sur le DDT et le DDD. 5) L'agitation d'échantillons d'eau contenant du γ-chlordane incubés en l'absence de sédiments n'a pas d'effet sur sa disparition, mais la présence de 0.01% d'un surfactant non ionique la retarde presque indéfiniment.

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Estimates for the global transport of persistent chemicals from points of release via different routes, such as sewage outfalls, surface run-off, rivers, and the atmosphere, are necessary to make predictions about their potential impact, especially upon the marine environment, as the open ocean has been claimed to be the ultimate sink for residues of persistent chlorinated hydrocarbon compounds (National Academy of Sciences 1971). However the means by which these chemicals are moved from points of release to the open ocean are still not well understood. Oloffs et al. (1972) recently studied the fate and behavior of several chlorinated hydrocarbons in three natural waters in the laboratory under nearly natural conditions and suggested several possibilities which may contribute to global distribution, and redistribution, of these compounds: (1) If present in natural waters at concentrations approaching or exceeding their solubilities, they tend to accumulate at the water–air interface whence they evaporate into the atmosphere at rates exceeding those explainable by codistillation. For example, 30–40% of some compounds were lost from water within 3 days and recovered quantitatively from the atmosphere. On the other hand, over 90% were still in the water after periods as long as 4 weeks if the treated water samples were kept in tightly stoppered vessels under otherwise identical conditions. (2) If occurring at concentrations below their water solubilities, these compounds tend to remain evenly distributed in the water, and the rate of transfer from the water into the atmosphere will be limited to that of codistillation. (3) The presence of materials promoting even

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distribution of chlorinated hydrocarbon compounds within water seems to decrease their transfer into the atmosphere.

The purpose of this investigation was to gain further insight into factors prevailing under natural conditions and their effects on the behavior of chlorinated hydrocarbon residues in natural waters.

Materials and Methods

Water samples were removed from the Fraser River and Georgia Strait as described (Oloffs et al. 1972). At the same time, several kilograms of bottom sediments were taken from the same sampling sites with an Ekman Grab and transferred to the laboratory in separate containers. The water temperatures at sampling were between 10 C and 15 C. Incubations in the laboratory were carried out at 13 C.

Experiment 1

One hundred-gram aliquots of each bottom sediment (Fraser River and Georgia Strait) were each placed into individual 500-ml Erlenmeyer flasks to give a layer of even thickness. Then a 150-ml portion of water was carefully added to each flask so that the layer of sediment was not disturbed. Immediately prior to this, the chlorinated hydrocarbon compounds were added to the water samples as described (Oloffs et al. 1972). The flasks were then plugged with silanized glass wool and incubated for 6 and 12 weeks at which times analyses were done (Table 1).

Prior to analysis, the water was slowly decanted so that all sediment remained in the flask. The water samples and the glass wool plugs were then analyzed separately as described (Oloffs et al. 1972). Each bottom sediment was extracted by blending with 200 ml of hexane and acetone (1/1, v/v), and the extract treated, cleaned, and analyzed as described by Oloffs et al. (1971).

Experiment 2

Sediments were placed into individual flasks as described (100 g per flask), stoppered with glass wool, and autoclaved for 45 min. Water samples (approx 1000 ml) were autoclaved separately and, after cooling, were treated to yield 0.025 ppm of either DDT, DDD, or lindane. After thorough shaking, 150-ml aliquots were added slowly to the flasks containing the autoclaved sediments so that the sediment layer remained undisturbed. These manipulations, following autoclaving, were carried out in a sterile transfer chamber. Nonautoclaved samples were prepared as in Experiment 1. Incubation and analyses were performed as described above (Table 2). Bacterial counts were determined by using nutrient agar (Difco). Inoculated plates were incubated at 15 C for 96 hr.

Experiment 3

Twenty 150-ml samples of Fraser River water were measured into 500-ml Erlenmeyer flasks without bottom sediment. To 10 of these, 15 mg of the nonionic surface-active agent Triton X-100 were added. After thorough mixing, all 20 samples were treated with γ-chlordane as described. The flasks were then stoppered with glass wool and incubated for up to 12 weeks at 13 C. One half of the samples containing both surfactant and γ-chlordane, and one half of those treated with γ-chlordane only, were swirled once every 24 hr during the incubation period. The other half was incubated without agitation or handling (Table 3). Water samples and glass wool plugs were analyzed individually after 0, 6, and 12 weeks.

Results and Discussion

With one exception, namely that of lindane in Georgia Strait water, all detectable residues had moved from the water into the underlying sediments after 6 and 12 weeks. No residues could be extracted from the glass wool plugs or the water samples (Table 1). These findings demonstrate the fundamental influence of bottom sediments on the fate and behavior of chlorinated hydrocarbons in natural waters (and possible pitfalls of laboratory models for studying these). Oloffs et al. (1972) had shown earlier that α-and γ-chlordane, DDT, and Aroclor 1260 (but not lindane) escaped from natural waters into the atmosphere by evaporation if incubated without bottom sediments under conditions otherwise similar to those used in the current study. They demonstrated that the movement of the compounds from the waters, held in Erlenmeyer flasks stoppered with silanized glass wool plugs, into the atmosphere appeared to be a two-step process. Initially, the compounds sublimed onto the glass wool, which always contained some of them after evaporation from the water–air interface; later they evaporated into the atmosphere.

Evidently, if present in natural waters not in contact with their natural bottom sediments, some chlorinated hydrocarbons tend to move towards, and accumulate at the water–air interface as suggested by Acree et al. (1963), and then evaporate into the atmosphere (Oloffs et al. 1972). The results of this investigation suggested that the presence of bottom sediments can not only prevent escape from water into the atmosphere, it can also prompt movement from the water into the sediment. Conceivably, the height of the water column (depth of water body) may influence the effects of these opposing forces: while in these experiments the water was only 3–4 cm deep, natural waters run deeper — especially still ones. But Green et al. (1966), who determined chlorinated hydrocarbon residues in approximately 1000 samples of natural waters collected throughout the USA found a lower incidence of residues at, or just below river impoundments, suggesting that stopping water flow,

i.e. agitation, may be a more important factor than depth of water.

Most of the lindane could not be recovered after 3, 6, and 12 weeks (Table 1). Quite likely, it was metabolized by microorganisms in the sediments since 96% were recovered from samples whose sediments and waters had been autoclaved prior to treatment and incubation (Table 1 and 2). Again, this brought out the interesting and profound effects bottom sediments can have on the fate of chlorinated hydrocarbon pollutants in the overlying waters: if incubated in Fraser River or Georgia Strait waters (nonsterile!), without the corresponding sediments being present, over 95% of lindane added remained in the water, even after 12 weeks (Oloffs et al. 1972).

One of the autoclaved lindane samples was not sterile at the time of analysis. Both the water and the sediment from this one flask yielded higher bacterial counts than the corresponding nonautoclaved samples. Still, more lindane was recovered from this flask than from the other three of that group, namely 99.2% (14.1% from the water and 85.1% from the sediment). Evidently, the bacteria in this flask were unable to metabolize lindane. Either this sample was contaminated after autoclaving, or autoclaving selectively killed those bacteria capable of utilizing lindane.

While autoclaving had a significant effect on the fate of lindane (see above), its effect was not very marked on both the movement from water into bottom sediment as well as on the disappearance of DDT and DDD (Table 2). The conversion of some DDT to DDD in Fraser River sediment, observed

TABLE 1. Fate of 0.025 ppm chlorinated hydrocarbon compounds in 150-ml water samples from Fraser River and from Georgia Strait in the presence of bottom sediments from same sources, incubated at 13 C. No residues could be detected on the silanized glass wool plugs used to stopper flasks during incubation. Controls (0–12 weeks) were free from detectable residues.

		Recovery in %			
	Incubation time	Fraser River		Georgia Strait	
Compound	(weeks)	Water	Sediment	Water	Sediment
α-chlordane	0	100	0	100	0
	6	0	90.0	0	69.3
	6	0	87.9	0	81.7
	12	0	79.7	0	78.5
	12	0	86.2	0	69.3
γ-chlordane	0	100	0	100	0
	6	0	98.8	0	65.8
	6	0	86.6	0	61.0
	12	0	93.3	0	62.7
	12	0	85.1	0	63.8
Lindane	0	100	0	100	0
	6	0	6.9	15.1	32.3
	6	0	8.8	19.1	27.7
	12	0	3.2	4.6	10.2
	12	0	2.9	6.2	9.7
DDT	0	100	0	100	0
	6	0	60.6(+2.8)[a]	0	68.6
	6	0	51.3(+5.7)[a]	0	73.7
	12	0	11.9(+16.2)[a]	0	80.5
	12	0	26.1(+13.0)[a]	0	82.0
PCB[b]	0	100	0	100	0
	6	0	70.4	0	70.6
	6	0	67.1	0	71.1
	12	0	72.8	0	80.7
	12	0	74.7	0	65.6

[a]DDD in % of applied DDT in brackets.
[b]Aroclor 1260 applied at 0.1 ppm.

in Experiment 1 (Table 1), did not occur in Experiment 2 (Table 2) although water and sediment for the latter were obtained from the same location.

Since rapid differential distribution of organochlorines within water has been shown to result in accumulation at the water–air interface and disappearance from the water if sediments are not present (Acree et al. 1963, Oloffs et al. 1972), it may be assumed that the presence of surface-active agents (=emulsification) and agitation (=mixing) will retard this process. Data in Table 3 indicate that agitation had no effect. But they show that the presence of a surfactant inhibited this movement significantly, so that the behavior of such compounds in sewage outfalls or in water bodies near cities may be quite different from that in other natural waters.

The results of this investigation suggest that a priori conclusions regarding the quantities of persistent chlorinated hydrocarbons present in

TABLE 2. Effect of autoclaving on fate of 0.025 ppm DDT, DDD, or lindane in 150-ml water samples from Fraser River incubated in the presence of bottom sediment at 13 C.

Compound	Incubation time (weeks)	Number of samples	Recovery in %			
			Water		Sediment	
			Mean	Range	Mean	Range
			Not Autoclaved			
DDT	0	2	100		0	
	12	4	T (2)[a]		65.7	60.4–71.7
DDD	0	2	100		0	
	6	4	T (1)		70.8	61.0–80.3
	12	4	0.8	0–2.8	60.2	54.9–65.5
Lindane	0	1	100		0	
	3	2	2.1	1.9–2.2	21.2	20.8–21.6
			Autoclaved			
DDT	0	2	100		0	
	12	8	5.9	0–16.0	70.1	56.5–77.5
DDD	0	2	100		0	
	6	8	10.2	2.9–23.0	76.9	64.9–90.4
	12	8	4.7	0–10.6	69.7	62.2–77.1
Lindane	0	1	100		0	
	3	4	14.7	13.3–17.9	81.2	76.2–85.1

[a]T: Trace (less than 1% of applied); brackets: number of samples with trace.

TABLE 3. Effects of agitation and surfactant on fate of 0.025 ppm γ-chlordane in 150-ml samples of Fraser River water at 13 C. Recovery in percent of applied.

Time (weeks)	Samples swirled by hand once per day						No agitation					
	No surfactant			0.01% Triton x-100			No surfactant			0.01% Triton x-100		
	Water	Glass wool	Total	Water	Glass wool	Total	Water	Glass wool	Total	Water	Glass wool	Total
0	100	0	100	100	0	100	100	0	100	100	0	100
6	37.0	1.1	38.1	93.6	0	93.6	34.2	8.3	42.5	94.4	0	94.4
6	20.6	3.8	24.4	92.7	0	92.7	38.4	8.3	46.7	87.6	0	87.6
12	4.6	0	4.6	82.9	0	82.9	6.0	0	6.0	75.1	0	75.1
12	13.8	1.2	15.0	79.1	0	79.1	less than 1%			71.4	0	71.4

the open ocean environment may well be misleading if based on levels found, or quantities manufactured and used elsewhere. For instance, residues found in a river near an intensely farmed area may partition into bottom sediments or be metabolized instead of reaching the marine environment. Residues originating from city sewage outfalls together with detergents, on the other hand, may well present a problem.

Acknowledgments

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Fish Viruses: Isolation and Identification of Infectious Hematopoietic Necrosis in Eastern North America

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Infectious hematopoietic necrosis virus (IHNV) was isolated from diseased fingerling rainbow trout (*Salmo gairdneri*) from a hatchery in West Virginia. Clinical signs, histopathologic findings, and origin of eggs provided a basis for diagnosis, and virus was isolated and presumptively identified by plaque characteristics. Serum neutralization tests provided positive identification of the agent as IHNV, and electronmicroscopy showed its rhabdovirus morphology. Experimental infections resulted in signs of IHNV and death; test fish had characteristic histopathologic alterations and appropriate virus titers. This is the first completely documented occurrence of IHNV beyond the Pacific Northwest.

WOLF, K., M. C. QUIMBY, L. L. PETTIJOHN, AND M. L. LANDOLT. 1973. Fish viruses: isolation and identification of infectious hematopoietic necrosis in eastern North America. J. Fish. Res. Board Can. 30: 1625–1627.

Nous avons isolé le virus de la nécrose hématopoiétique infectieuse (IHNV) chez des alevins de truite arc-en-ciel (*Salmo gairdneri*) dans un établissement de pisciculture de la Virginie de l'Ouest. Les symptômes cliniques, des observations histopathologiques et l'origine des oeufs ont servi de base au diagnostic, et le virus a été isolé et présomptivement identifié par les caractéristiques des plaquettes. Des tests de neutralisation du sérum ont fourni une identification positive de l'agent pathogène comme étant IHNV, et l'examen au microscope électronique a révélé une forme de rhabdovirus. Des infections expérimentales ont causé l'apparition des symptômes de IHNV et la mort; les poissons expérimentaux montrèrent des modifications histopathologiques caractéristiques et des titres appropriés de virus. C'est le premier cas complètement documenté de IHNV au-delà du Pacifique Nord-Ouest.

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INFECTIOUS hematopoietic necrosis (IHN), an acute viral disease of young sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*) has occurred almost exclusively in the Pacific Northwest. Recent reports on the disease and its history, morphology and biophysical properties of the virus, and probable synonomy of IHN with Oregon sockeye virus disease and Sacramento River chinook disease were published by Amend et al. (1969); Amend and Chambers (1970a, b); McCain et al. (1971); Darlington et al. (1972); and Amend et al. (1973).

On the basis of epizootiological data, cytopathologic changes in infected cell cultures, and histologic findings, IHN was diagnosed in 1969 among rainbow trout in South Dakota (unpublished diagonostic findings). Plumb (1972) reported viral disease among rainbow trout in Minnesota during 1969; he employed epizootiologic data, clinical signs, histologic examination, experimental infections, and several biophysical properties of the virus to arrive at a conclusion that the etiologic agent was a virus similar to IHNV. Since a pathognomonic feature of IHN is degeneration and necrosis of granular cells in the wall of the alimentary canal (Yasutake 1970), it is not likely that there was an error in diagnosis. Nevertheless, the most accurate determination of an infectious disease necessitates isolation and identification of the causal agent by serologic procedures.

The purpose of this report is to document the occurrence of IHN in eastern North America and to provide details which completely identify the virus.

Case History and Clinical Findings

During July 1972, 9-month-old fingerling rainbow trout (*Salmo gairdneri*), 10–13 cm in total length at a commercial facility in West Virginia showed signif-

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icant mortality and ultimately about a 60% loss during a 2-week period. The fish originated from eggs that had been shipped from a source in the Pacific Northwest. The eggs had not been disinfected at the point of receipt. Water chemistry was not known. Temperature was thought to be 10 ± 2 C.

Representative specimens were submitted for diagnostic examination. Fish were exophthalmic and dark. Parasites were not found, but an unidentified myxobacterium was cultured from the kidneys. Considering the source, signs, and species, infectious hematopoietic necrosis (IHN) was also suspect. Histopathologic examinations revealed severe and widespread necrosis of renal hematopoietic tissue and moderate focal necrosis of kidney tubules, zonal necrosis of liver, degenerative changes in walls of the digestive tract, and localized pancreatic necrosis. The findings provided pathologic diagnosis of IHN. Bacteria-free filtrates were prepared from 10% tissue homogenates and inoculated into tube cultures of RTG-2 cells and incubated at 15 C while homogenates were stored at –20 C.

Virus Isolation and Identification

The original homogenate which had been stored at –20 C was thawed, passed through a 0.45-μm membrane filter, serially diluted in tenfold steps, and assayed for infectivity on 60-mm plate cultures of RTG-2 cells incubated at 15 C. One set of plates held liquid medium while a second set was used for plaque assay with a two-phase gel–liquid overlay (Wolf and Darlington 1971). Plaques appeared by the 4th day, and plates were fixed and stained on the 5th day. Cultures in liquid medium also showed cytophathic effects; the cultures were allowed to proceed to complete cell destruction then the medium was pooled and stored at 4 C.

Plaques were the type produced only by IHN virus (Wolf and Quimby 1970). There was a pile-up of cells at plaque margins and coarsely discontinuous cellular debris within the opening of the plaque. Peripheral cells showed margination of chromatin. As measured in a single plate series there were 2.6×10^3 plaque forming units (PFU)/g of fish homogenate.

The pooled medium from liquid cultures was used for serum neutralization tests, an in vivo infectivity test, and infecting additional cultures to be examined by electronmicroscopy.

Neutralization Test

Plaque reduction testing was carried out using normal rabbit serum as control and rabbit immune serum prepared against Egtved virus[1], against cloned IHN virus isolated from chinook salmon (*Oncorhynchus tshawytscha*), and a polyvalent mixture against five antigenically separated strains of infectious pancreatic necrosis virus (IPNV). Equal volumes of virus and antisera were reacted for 30 min at 15 C, then adsorbed onto plate cultures of RTG-2 cells. Cultures were overlaid for plaquing then incubated at 15 C.

As seen in Table 1, only anti-IHNV serum neutralized the West Virginia virus isolant. At a serum dilution of 1:50 there was a 100% reduction of at least 2900 PFU's and antisera prepared against the other two salmonid viruses were without neutralizing effect.

In Vivo Infectivity Trial

Five-month old rainbow tront fingerlings[2], 6–7 cm total length, 4–5 g, from a lot that had been assayed virologically and found to be free of virus were acclimated at 6–8 C in water whose chemistry has been reported (Warren 1963). An aliquot of the West Virginia IHNV isolant was divided and a portion heated at 56 C for 30 min and a portion was left unheated. Five fingerling trout were injected intraperitoneally with 5×10^5 PFU of live virus in 0.03 ml, and five fish received the same amount of heat-killed virus.

Control fish remained normal throughout 2 weeks observation. Fish that received live virus showed clinical signs of IHN on the fifth day postinoculation, and the first death occurred on the 6th day. A moribund specimen was taken for histologic examination, and the remaining three died on the 9th, 10th, and 13th days, respectively.

Victims were all lethargic, abnormally dark, had distended abdomens, and several trailed a long, prominent mucoid rectal pseudocast, presumably of mucosal origin. Exophthalmia was absent. Internally, liver and kidneys were very pale and the latter had focal hemorrhages. Histologic examination showed some of the pathologic changes associated with IHN. Renal hematopoietic tissue and pancreatic tissue were necrotic. Hemor-

[1]Serum obtained from Dr P. E. Vestergard Jørgensen, State Veterinary Serum Laboratory, Aarhus, Denmark.

[2]Biety's Resort, Washington, Strain.

TABLE 1. Results of plaque reduction serum neutralization testing of West Virginia suspect IHN virus.

Rabbit serum	Dilution	Virus dilution	Mean PFU's[a]
Normal	1:50	10^{-2}	29
Polyvalent anti-IPNV[b]	1:100	10^{-2}	51
Anti-Egtved virus[c]	1:100	10^{-2}	29
Anti IHNV[d]	1:50	10^{-2}	0
Anti-IHNV	1:50	10^{-1}	0
Anti-IHNV	1:50	10^{0}	0

[a]Plaque forming units.
[b]Titer against 100 PFU's homologous virus = 1:800 to 1:1000.
[c]Titer against 100 PFU's homologous virus = >1:2000.
[d]Titer against 40 PFU's homologous virus = 1:50.

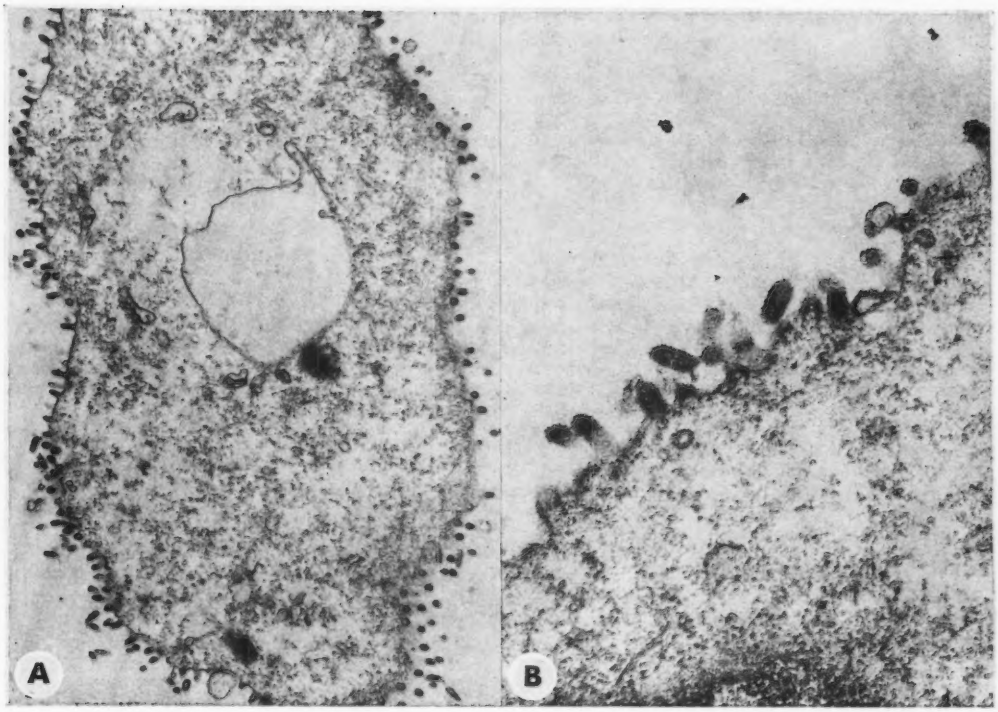

FIG. 1. Electronmicrographs of RTG-2 cells infected with West Virginia isolant of IHNV. (A) abundant bullet-shaped virions are budding from the cell membrane (×22,000), (B) bullet-shaped virions show cross-striations and longitudinal inner structure after maturation (×72,000).

rhage was prominent in ventral fin bases. Mucosal sloughing was evident in some areas, but at most the gut wall showed degenerative change rather than clear-cut necrosis.

Two dead specimens were assayed for infectivity. There were 4.1×10^6, and 8.2×10^6 PFU's/g, respectively. On the basis of mean fingerling weight (4.5 g), this represented a 37- and a 74-fold increase, respectively, in the amount of infective virus injected.

Electronmicroscopy

RTG-2 cell cultures were infected with the West Virginia virus isolant, incubated for 2 days at 15 C fixed in Millonig's buffered formalin at *p*H 7.3, embedded, sectioned, and stained as reported elsewhere (Wolf and Darlington 1972).

Electronmicrographs showed abundant rhabdovirus identical in size and morphology to IHNV (Fig. 1). Virus was not found intracellularly but maturing at cell membranes.

Conclusions

IHNV has been unequivocally isolated and identified in eastern North America. Identification is based upon disease signs in both the diagnostic specimens and experimentally infected test rainbow trout, upon electronmicroscopy, plaque characteristics, and most critically, upon serum neutralization test with specific rabbit immune serum.

Considering the amount of virus quantified in the original diagnostic specimens, it does not appear justified to attribute mortality solely to the virus; the amount was considerably less than is usually encountered in epizootics. Secondly, a bacterial pathogen was also present in kidneys of the diagnostic specimens.

Either as a contaminant or as an infection, epizootiological evidence favors the interpretation that IHNV was transported from the Pacific Northwest with eggs; we know that they were not disinfected upon receipt. Although it is less tenable, we cannot absolutely rule out a possibility that the virus was present at the hatchery before the subject eggs were received.

INHV has been documented in Minnesota (Plumb 1972) but, based upon histologic examination, it has also occurred in Montana (C. Smith personal communication) and South Dakoat. A characteristic epizootic occurred in Viriginia 2 years ago and IHNV-like cytopathologic changes observed by two of us (K. Wolf and M. C. Quimby unpublished data) in cultures of RTG-2 cells. Circumstances at the time prevented a follow-up study, and the agent was lost.

The significance of the work reported here is that it documents man's spread of a fish virus far beyond its previously known range. Since this work was begun, we have been shown evidence that IHN has been diagnosed in rainbow trout in Japan (T. Kimura personal communication).

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Beverton-Holt Model of a Commercial Fishery: Optimal Dynamics

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The problem of optimal regulation of a fishery is discussed. Of special interest is the problem of regulating an overexploited fishery by reducing effort to allow the fish population to build up to a suitable level.

We first argue that the problem requires an economic analysis based on the concept of maximization of present value. From this concept we then deduce a simple, general rule, the "Fisher Rule," which we subsequently use to determine optimal exploitation. Among the principal results are the following: (a) an optimal mesh-size is determined, which, because of the discounting of future revenues, is smaller than the size corresponding to maximum sustainable yield; (b) the optimal recovery policy for an overexploited fishery is deduced; it consists of a fishing closure permitting the fish population to reach an optimal age; (c) the optimal development of an unexploited fishery is deduced; an initial development stage characterized by large landings and profits is rapidly transformed into a situation of optimal sustained yield, in which both landings and profits may be significantly reduced; (d) the optimal policy is deduced for a fishery in which gear limitations are impractical; the result may be a strongly unstable fishing industry; (e) the effect of high discount rates, which might be employed by private fishing interests, is discussed; such rates may result in overfishing similar to the case of a common-property fishery.

CLARK, C., G. EDWARDS, AND M. FRIEDLAENDER. 1973. Beverton-Holt model of a commercial fishery: optimal dynamics. J. Fish. Res. Board Can. 30: 1629–1640.

Nous traitons de la question du contrôle optimal d'une pêcherie. Un cas d'intérêt particulier est le réglage d'une pêcherie surexploitée par réduction de l'effort afin de permettre à la population d'atteindre un palier convenable.

Nous soutenons tout d'abord que le problème exige une analyse économique fondée sur l'idée de la maximisation de la valeur présente de la pêcherie. Nous déduisons de ce concept une règle simple et générale, appelée "règle de Fisher," qui nous permet de préciser par la suite l'exploitation optimale. Les principaux résultats sont: (a) détermination de la grandeur optimale de la maille; à cause de l'escompte de revenus futurs, cette maille est plus petite que celle qui correspond au maintien constant des prises maximales; (b) définition d'une politique optimale de redressement d'une pêcherie surexploitée; ceci comporte une suspension de la pêche pour permettre aux poissons d'atteindre un âge optimal; (c) détermination du développement optimal d'une pêcherie vierge; une phase initiale de développement, caractérisée par des débarquements et des profits élevés, se transforme rapidement en une situation de rendement optimal soutenu, avec débarquements et profits appréciablement diminués; (d) mise au point d'une stratégie optimale pour une pêcherie où il n'est pas pratique d'imposer des restrictions sur l'équipement; il peut en résulter une industrie très instable; (e) discussion des conséquences du taux d'escompte élevé que peuvent adopter des intérêts privés et qui pourraient conduire à une surexploitation semblable à celle d'une pêcherie de propriété publique.

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THE subject of this article is the optimal management of a commercial fishery, whether privately or publicly owned, and the first question that must be faced is the meaning of the word "optimal." The most obvious and most frequently encountered interpretation of optimality is embodied in the concept of maximum sustained yield. This interpretation has much to recommend it, particularly in view of the increasing prevalence of severe overexploitation in fisheries and other renewable resources.

Nonetheless maximum sustained yield has serious limitations as a criterion for resource management.

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Consider, for example, the case of a heavily overexploited fishery. It is clear that any management program designed to increase yield will involve at least a temporary reduction in landings before the desired increase is achieved. Conservation inevitably requires some degree of current sacrifice to obtain future benefits, so that we are immediately faced with the basic economic question of "trade-offs" between income and investment.

The concept of maximum sustained yield, being a noneconomic concept, has nothing to contribute to this basic economic problem. Nor does it have anything to say about the development of a virgin resource stock, since it is not a dynamic concept — it specifies only the final state, not the path by which such a state is reached.

A possible suggestion for the optimal development — or recovery — of a fishery would be that the level of maximum sustained yield should be reached as rapidly as possible. An overexploited fishery, for example, would be closed until the stock had recovered to the desired level, as is currently the situation in the blue whale fishery.

Can such a policy be considered optimal in every case? If, for example, there are fishermen whose very survival depends upon the fishery in question, or fishing companies holding large investments in boats and gear, would the increased revenues to be obtained from building up the fishery justify a scheme of temporary compensation?

Another question closely related to the above concerns a single company (or public agency) that owns complete property rights in a certain fishery. It can sell its rights to another company, or it can operate the fishery itself. If it sells, what is a fair price? If it operates the fishery itself, what management scheme will it adopt?

All these questions can be treated by considering not the maximization of physical yield, but rather the maximization of economic benefits. Specifically, a rational management policy, whether private or public, will attempt to maximize the total "present value" of all profits, present and future, from the resource in question.

Such a definition, while still open to question on various technical grounds (such as the problem of uncertainty, or the distinction between private and social discount rates), is at least a complete definition in the sense that it determines in principle the entire management program. It provides the basis for cost–benefit analysis by means of which trade-offs of all kinds can be quantified; it also determines a fair selling price, or economic value, of the resource, namely the maximum present value itself.

The definition, however, raises a critical question: what is the correct discount rate to utilize in the computation of present values? This question is beyond the scope of this paper (see Baumol 1968), and the discount rate λ will therefore be taken as "given." The cases of small discount rates ($\lambda \simeq 0$) as well as large ones ($\lambda \to \infty$) are particularly interesting, since (as we shall explain) they correspond, respectively, to the maximization of sustained economic yield, and the "dissipation of economic rent" as described by economists for the case of common-property fisheries (Gordon 1954).

In the remainder of this article we shall first introduce and analyze our basic model, following Beverton and Holt (1957), for the case of a single year-class of fish. The analysis will then be extended to the case of conglomerate year-class structures. The entire analysis is based on a single rule that we call the "Fisher Rule" after the economist I. Fisher (1930).

Mathematically speaking, the problems to be analyzed are problems in optimal control theory, which can be dealt with by means of the maximum principle of L. S. Pontrjagin (1964). Rigorous mathematical details, however, are relegated to the Appendix, and the main body of the paper is devoted to the discussion and intuitive interpretation of the results of the theory.

Single Year-Class Model

In fisheries economics, and in the literature of fisheries management generally, there are two basic and quite different types of models, the "logistic" models in which the population is treated as a homogeneous quantity, and the more refined models based on "year-class" structures.

Logistic models are normally based on a differential equation of the form $dN/dt = f(N)$, for the total population $N = N(t)$. The function $f(N)$ thus combines the effects of recruitment, growth, and natural mortality into a single relationship between the population N and its net growth rate dN/dt. Fishing in such a model is assumed to counteract natural growth, and the equation

$$\frac{dN}{dt} = f(N) - h(t)$$

is taken to describe the combined process. Here $h(t)$ the "fishing rate," is the control variable for optimization problems. The economic equilibrium analysis of this model is due to Gordon (1954) and Scott (1955). A complete dynamic analysis, based upon present-value maximization, has recently been given by Clark (1973a,b).

In the Beverton-Holt model, on the other hand, recruitment is taken to be constant, and the population numbers are reduced instead by a constant mortality rate. Thus, if $x = x(t)$ denotes the number of fish in a given year-class (recruited at time $t = T$), then we suppose that under natural conditions we have

$$\frac{dx}{dt} = -Mx,\ x(T) = R \tag{1}$$

where the recruitment R and the natural mortality rate M are both positive constants. A variable "fishing mortality" $F(t)$ is assumed to affect the population of any particular year-class $x(t)$ by simply increasing the natural mortality:

$$\frac{dx}{dt} = -(M + F(t))x \tag{2}$$

This function $F(t)$ is now the control variable; it specifies the age at which fish should be caught and the intensity of fishing at each age.

The model also accounts for the growth in weight of the fish, by means of a function $w(t)$ representing the (average) weight of a fish of age t. We shall assume that $w(t)$ is an increasing, bounded function of t, with $w(t) \to w_\infty$ as $t \to \infty$. For technical reasons, we shall also assume that $w'(t)/w(t)$ is decreasing. The latter is a sort of convexity assumption; it is valid for any concave growth curve $w(t)$ and also for the standard von Bertalanffy curve $w(t) = a(1-e^{-kt})^3$.

Restricting our attention henceforth to the Beverton-Holt model, we shall begin our analysis by considering the problem of maximizing the present value of the harvest from a single year-class, recruited at time $t = 0$.

Let $B(t)$ denote the biomass of the given year-class:

$$B(t) = x(t)w(t). \tag{3}$$

From (2) we have $B' = x'w + xw' = -(M + F)xw + xw'$, so that

$$B' = B\left(\frac{w'}{w} - M - F\right). \tag{4}$$

In the time interval from t to $t + dt$ the yield in biomass is given by

$$Y(t)dt = F(t)x(t)w(t)dt = F(t)B(t)dt.$$

This completes the description of the biological aspects of our model, and we now turn to its economic aspects.

First we shall assume a fixed price p per unit weight of fish. Next we assume that fishing costs are proportional to fishing effort, which in turn is proportional to the fishing mortality rate $F(t)$. This assumption, sometimes referred to as "mass contact fishing technology," has a simple probabilistic interpretation in terms of the expected time to catch one fish from a population of x fish, uniformly distributed, see Clark (1973a). (For the sake of clarity we shall henceforth refer to $F(t)$ itself as fishing effort rather than fishing mortality.) Consequently the net revenue, or profit, derived during the time interval from t to $t + dt$ is given by

$$\begin{aligned} R(t)dt &= [pY(t) - cF(t)]dt \\ &= F(t)[pB(t) - c]dt \end{aligned} \tag{5}$$

where c is a constant denoting the cost of a unit fishing effort.

If $\lambda > 0$ represents the given (instantaneous) discount rate, then the total present value of all profits is given by the expression

$$PV = \int_0^\infty e^{-\lambda t} F(t)[pB(t) - c]dt \tag{6}$$

The problem (for a single year-class) is therefore to determine the fishing-effort function $F(t) \geq 0$ that maximizes the expression (6), where $B(t) = x(t)w(t)$ and $x(t)$ satisfies the basic equation (2). A special case, as we shall see, is the problem considered by Beverton and Holt of simply maximizing the total revenue.

In the absence of fishing, the biomass satisfies the equation

$$B'(t) = B(t)\left(\frac{w'}{w} - M\right); \tag{7}$$

we shall refer to this as the "natural biomass equation." Since w'/w is assumed to be decreasing, we see that the natural biomass increases to a maximum value, which occurs at time t_0 determined by

$$\frac{w'(t_0)}{w(t_0)} = M \tag{8}$$

and subsequently $B(t)$ decreases — see Fig. 1. If equation (8) has no solution, then the biomass $B(t)$ has its maximum value when $t_0 = 0$. Notice that even if the population has been reduced by fishing, once fishing ceases the biomass will continue to satisfy equation (7), and will therefore follow the corresponding curve of Fig. 1. This fact plays an important role in our description of optimal fishing.

According to equation (5) the quantity

$$V(t) = pB(t) - c \tag{9}$$

represents the net revenue generated by a unit fishing effort per unit time. The quantity $V(t)$ will be called the "net biovalue" of the fish population at time t.

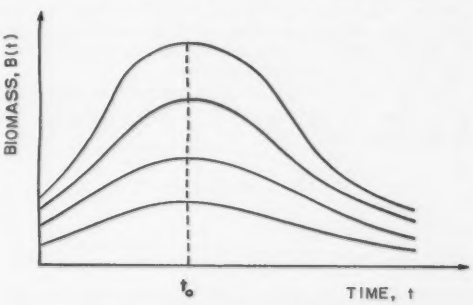

FIG. 1. Natural biomass curves.

In the absence of fishing, the natural rate of change in the net biovalue is

$$V'(t) = pB'(t) = pB(t)(\frac{w'}{w} - M) \qquad (10)$$

where we have used the natural biomass equation (7). We can now describe the optimal fishing policy.

At any given time t, the decision must be made as to the optimal fishing effort $F(t)$. This decision does not depend upon the past history of the fishery, but only upon the existing net biovalue $V(t)$ and its potential future growth, given by equation (10). First of all, it is clear from the expression (6) and the differential equation (2) that

$$F(t) = 0 \text{ unless } V(t) > 0 \qquad (11)$$

for otherwise there would result an unnecessary negative contribution to present value. On the other hand, if $V(t) > 0$, the decision to fish or not to fish will depend on a comparison of the rate $V'(t)$ at which the net biovalue is changing as a result of natural growth, with the rate of growth of an equivalent monetary investment at the given rate of interest λ. Specifically, if $V'(t) > \lambda V(t)$ (with $V(t) > 0$) then it is better to postpone fishing, since the net biovalue of the fish population is increasing faster than its monetary equivalent. Conversely, a population whose net biovalue is growing more slowly than its monetary equivalent should be harvested and converted into cash value. Thus for $V(t) > 0$, we have the following rule:

Fisher Rule — The optimal fishing effort satisfies

$$F(t) = \begin{cases} 0 & \text{if } V'(t) > \lambda V(t) \\ F_{max} & \text{if } V'(t) < \lambda V(t) \end{cases} \qquad (12)$$

where F_{max} denotes the maximum possible fishing effort, and $V'(t)$ is given by equation (10). Marginal rules of this kind are common throughout investment theory (see Hirshleifer 1970).

The Fisher Rule, as we shall see later, does not necessarily apply in all possible cases. It does apply, however, provided that the function $V'(t)-\lambda V(t)$ has at most one change of sign, as is the case in this section (but not in the next section). An elementary rigorous proof of the rule is given in the appendix.

To see how the Fisher Rule operates in our case, we consider the inequality $V'(t) > \lambda V(t)$, which by (10) is equivalent to

$$pB(t)(\lambda + M - \frac{w'}{w}) < c\lambda$$

This inequality is automatically satisfied for $\frac{w'}{w} > \lambda + M$, while for $\frac{w'}{w} < \lambda + M$ it is equivalent to the inequality

$$B(t) < \Phi_\lambda(t) = \frac{c\lambda}{p(\lambda + M - \frac{w'}{w})}. \qquad (13)$$

Note that the curve $\Phi_\lambda(t)$ is monotone decreasing (since $\frac{w'}{w}$ is) with a vertical asymptote at time t_λ determined by the condition[1]

$$\frac{w'(t_\lambda)}{w(t_\lambda)} = \lambda + M. \qquad (14)$$

If equation (14) has no solution t_λ, then there is no vertical asymptote. Note also that $\Phi_\lambda(t_o) = \frac{c}{p}$ where t_o is the time of "maximum natural biomass" determined by equation (8). These relationships are illustrated in Fig. 2, with time along the horizontal axis and biomass along the vertical (as in Fig. 1). The shaded area in Fig. 2 represents the "fishing zone," where both $V(t) > 0$ and $V'(t) < \lambda V(t)$, or (in terms of biomass) where $B(t) > c/p$ and $B(t) > \Phi_\lambda(t)$. The Fisher Rule requires maximum-intensity fishing in this zone. Similarly, the Fisher Rule requires zero fishing in each of the zones defined by the inequalities $t < t_\lambda$, $B(t) < \Phi_\lambda(t)$, $B(t) < c/p$. The boundary of the fishing zone is defined by the condition $V'(t) = \lambda V(t)$ (or $B(t) = \Phi_\lambda(t)$) subject to the restraint $V(t) \geq 0$ (or $B(t) \geq c/p$).

Let us now follow the biomass curve $B(t)$ corresponding to optimal fishing. Figure 2 shows the case in which the initial biomass lies in the no-fishing zone. In this case $B(t)$ will follow the natural biomass curve until that curve hits the boundary $B = \Phi_\lambda(t)$ of the fishing zone. If we assume that F_{max} is large, then the biomass curve under optimal fishing will never actually enter the fishing zone, since if it did so, the maximum fishing effort would immediately reduce biomass to the level $\Phi_\lambda(t)$. Optimal fishing could not reduce the biomass below this curve, for then fishing would cease, and the

[1]Our notation for t_o, t_λ, and T differs from that of Beverton and Holt (1957).

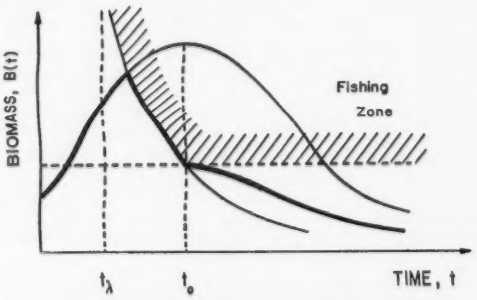

FIG. 2. Optimal fishing — the Fisher Rule.

natural growth would force $B(t)$ back towards $\Phi_\lambda(t)$. Consequently the optimal fishing effort maintains the biomass $B(t)$ precisely on the boundary curve $\Phi_\lambda(t)$, wherever possible.

Eventually, however, fishing reduces the biomass to the level of zero revenue, $V(t) = 0$. Since $\Phi_\lambda(t_0) = c/p$, it follows that optimal fishing just succeeds in reducing biovalue to the zero level at the exact instant when natural biomass reaches its maximum value. In other words, since the curve $\Phi_\lambda(t)$ is characterized by the "Fisher equation"

$$V'(t) = \lambda V(t),$$

at $t = t_0$ we have $V'(t_0) = V(t_0) = 0$.

At $t = t_0$ fishing therefore ceases, and the biomass subsequently follows the natural curve. In Fig. 2, the heavy curve shows the overall biomass curve $B(t)$ as affected by optimal fishing.

Although the optimal fishing policy just described is obviously too complex to be of much practical value in fisheries management, it does possess certain pertinent features. It shows for example, that regardless of the costs of fishing (which are difficult if not impossible to determine in practice), optimal fishing ta es place only when

$$t_\lambda < t < t_0$$

where t_λ is determined by equation (14). Indeed, optimal fishi g begins at some time $t^* > t_\lambda$ depending on the economic and biological parameters; for example, t^* increases if the cost parameter c increases.

Three particular cases, illustrated in Fig. 3, are especially easy to describe.

Suppose first that the costs of fishing are negligible, $c = 0$. Then $V'/V = B'/B = (xw'-Mxw)/xw = \lambda$ if and only if $t = t_\lambda$, so that the curve Φ_λ degenerates to the vertical line $t = t_\lambda$. Hence optimal fishing applies a maximum possible effort at the time $t = t_\lambda$ (Fig. 3a). (Figure 3a and b are drawn under the assumption that the maximum fishing effort F_{max} is large, so that the fish population can be rapidly reduced by fishing. The case of a limited maximum rate F_{max} has been studied by Goh (1972), who shows that (assuming $c = \lambda = 0$) optimal fishing will begin at the age t^* determined by the equation $B(t^*) = \int_{t^*}^{\infty} F_{max}\, B(t)dt$. It can be shown that $t^* \to t_0$ as $F_{max} \to +\infty$.)

Consider next the case of a zero discount rate, $\lambda = 0$. Then $V'/V = 0$ if and only if $t = t_0$, and the curve Φ_λ becomes the vertical line $t = t_0$. Optimal fishing applies a maximum effort at the instant t_0 of maximum natural biomass. Optimal fishing with no discounting of future revenues is therefore equivalent simply to the maximization of total revenue. This was the case considered in the book of Beverton and Holt (1957).

Finally we consider the limiting case of an infinite discount rate. From equation (13) it follows that $\Phi_\lambda(t) \to c/p$ as $\lambda \to \infty$. Fishing therefore takes place whenever $pB(t) > c$. Since the net biovalue never attains a positive value, the total present value is equal to zero in this limiting case. This is similar to the classical economic model of a common-property fishery (Gordon 1954), which predicts that zero "economic rent," i.e. zero net profits, will be derived by the competing fishermen. It has been observed previously by Clark (1973a,b) that the common-property fishery is equivalent mathematically to the limiting case of maximization of present value with an infinite rate of discount.

To explain this observation more fully, we remark that, in the case of a common-property resource, the individual exploiter can hold no confidence that the investment stock (i.e. the fish population) will remain intact. Whenever the resource has a positive net value, the danger exists that a competitor will harvest it. The

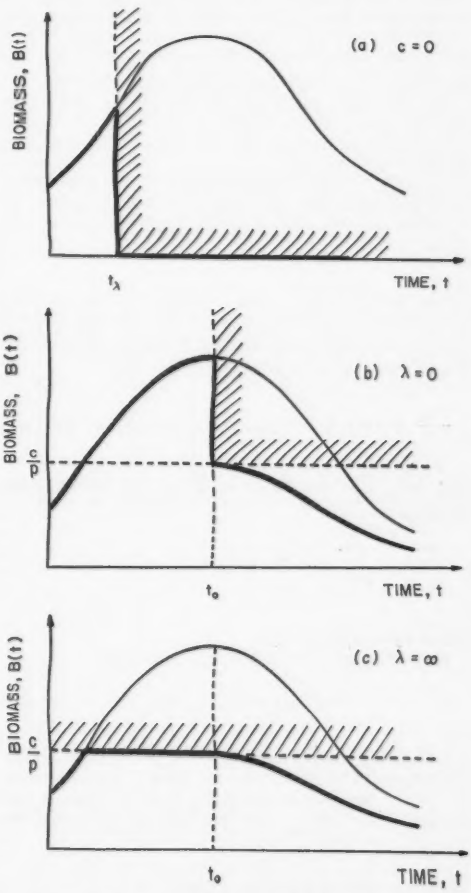

FIG. 3. Optimal fishing — special cases: (a) zero cost; (b) zero discount rate; (c) infinite discount rate

individual fisherman cannot afford to leave the investment to increase in value since he may thereby lose it entirely. Potential future revenues must therefore be discounted to zero since they cannot be realized in a competitive situation. The effective discount rate for common-property exploitation is consequently infinite.

Conglomerate Year-Classes

Most commercial fisheries do not exploit a single year-class. The Pacific halibut (*Hippoglossus* spp.) fishery, for example, normally covers an age-span from 7 to 18 years (Crutchfield and Zellner 1962).

Theoretical investigation of the optimal dynamics of conglomerate year-class structures presents many challenging difficulties. If the effort applied to each year-class could be selected independently, there would of course be no new problem — the optimal overall policy would simply consist of separate optimization for each year-class. Any practical fishing method, however, will generally affect many year-classes simultaneously.

It is fortunate, therefore, that the analysis of the preceding section, and in particular the Fisher Rule, can be extended to give considerable information regarding conglomerate age structures.

We begin by considering the case of two populations $x_1(t)$ and $x_2(t)$. The populations have initial values

$$x_i(T_i) = R_i, \quad i = 1,2$$

where T_i is the time of recruitment. The fishing effort $F(t)$ is assumed to affect both populations equally; this assumption will be modified later. We therefore obtain the following mathematical model.

$$\frac{dx_i}{dt} = -(M + F(t))x_i, \quad x_i(T_i) = R_i \; (i = 1,2) \qquad (15)$$

For simplicity we take $T_1 = 0$. The present-value expression is

$$PV = \int_0^\infty e^{-\lambda t} F(t)[pB_1(t) + pB_2(t) - c] \, dt \qquad (16)$$

where $B_i = x_i(t)w(t-T_i)$.

As in the previous section, the expression

$$V(t) = pB_1(t) + pB_2(t) - c \qquad (17)$$

will be called the "net biovalue" of the combined population, and $V'(t)$ will denote the natural rate of change of $V(t)$ in the absence of fishing. It can be shown (see appendix) that the optimal fishing effort F(t) satisfies the following weak form of the Fisher Rule:

$$F(t) = \begin{cases} 0 \text{ if } V'(t) > \lambda V(t) \\ 0 \text{ or } F_{max} \text{ if } V'(t) < \lambda V(t). \end{cases} \qquad (18)$$

The optimal effort F(t), however, cannot be unambiguously deduced from this rule. The mathematical reason for the difficulty lies in the fact that $V'(t)-\lambda V(t)$ may have several changes of sign, so that refraining from fishing even when $V'(t) < \lambda V(t)$ may result in increased profits at a later time.

There is one case, however, that can be analyzed in detail, namely the case in which costs of fishing are negligible: $c = 0$. Notice that in this case the present-value expression (16) depends linearly upon the biomasses $B_1(t)$ and $B_2(t)$. It follows that an optimal fishing policy must maximize the contribution from each population separately, at least inasmuch as separation can be achieved. Let us consider two concrete examples.

First we consider the case in which the two biomass curves are "nonoverlapping" (Fig. 4a). We know from the previous section that the maximum present value is obtained from the population B_1 by harvesting the entire population at an optimal age $t_{\lambda 1}$. Once the first population has been harvested, the second population is considered independently, and harvested at its corresponding optimal age $t_{\lambda 2}$, i.e. at time $t = T_2 + t_{\lambda 2}$. This argument is valid provided

$$t_{\lambda 1} < T_2 \qquad (19)$$

and we may take this inequality as the (economic) definition of "nonoverlapping."

Suppose next that the two population curves do overlap (Fig. 4b). In this case there is no incentive to harvest the first population before the time $t = T_2$ when the second population enters the fishery. For $t > T_2$, the complete Fisher Rule of the previous section can be used, thereby determining an optimal time t_λ ($t_{\lambda 1} < t_\lambda < t_{\lambda 2} + T_2$) at which both populations are harvested completely.

It is important to note that, in the case of overlapping year-classes, the optimal harvest policy for the combined population does not generally result in the optimal harvest for each year-class separately. The purpose of mesh-size limitations is precisely to increase the total harvest by allowing the escape of immature (x_2) fish while the mature (x_1) fish are being harvested.

Let us now consider a mesh-size limitation, which we shall assume simply prevents the capture of all fish less than some critical age T^*. Since fishing decisions are made only in terms of the fish available to the fishery, it is sufficient to consider the modified biomass curves (Fig. 5):

$$B_i^*(t) = \begin{cases} 0 & \text{if } t \le T_i + T^* \\ B_i(t) & \text{if } t > T_i + T^* \end{cases} \qquad (20)$$

It is easy to see now that the optimal age T^* is the same as the optimal age t_λ for a single year-class. To explain this conclusion, we note that for this choice, the modified biomass curves $B_i^*(t)$ are nonoverlapping according to the definition (19). Optimal fishing therefore harvests each population at the optimal age t_λ; by the mesh-size limitation, harvesting the first population has no effect on the immature fish of the second population.

It follows that optimal fishing for the combined population automatically maximizes the present value from each separate population. Obviously no better policy than this can exist!

To summarize the results of this section, we have shown that, if fishing costs are neglected, then optimal fishing requires the use of a mesh-size S corresponding to the optimal age t_λ determined by the condition

$$\frac{w'(t_\lambda)}{w(t_\lambda)} = M + \lambda.$$

Moreover, the optimal fishing policy harvests each population as it reaches the age t_λ.

If fishing costs are significant, then it appears that the optimal age T^* will be larger than t_λ, and optimal fishing will spread the effort more uniformly over the fishing season. As stated earlier, however, we have not been able to solve this problem in general.

Sequential Year-Classes

Most of the analysis of the previous section can be extended easily to the case of several year-classes. In this section we shall briefly consider the case of a continuing sequence (x_n) of year-classes, all assumed to belong to the same species. If no mesh-size limitation is in effect, then the appropriate mathematical model is as follows.

$$\frac{dx_n}{dt} = -(M + F(t))x_n \quad (t \geq T_n), \tag{21}$$

$$x_n(T_n) = R = \text{constant} \tag{22}$$

$$PV = \int_0^\infty e^{-\lambda t} F(t)[p \sum_{n=0}^{\infty} w(t-T_n)x_n(t) - c]\, dt \tag{23}$$

where R is a constant recruitment rate, T_n is the time of recruitment of the n^{th} year-class, and $w(t-T_n)$ represents the weight of a member of the n^{th} year-class at time $t > T_n$. Because $x_n(t)$ decreases at least as fast as e^{-Mt} the series appearing in (23) converges uniformly, and there are no problems with convergence of the integral.

As in the previous section, we shall consider only the case of negligible fishing costs, $c = 0$. The expression

$$V(t) = p \sum_{n=0}^{\infty} B_n(t)$$

again denotes the net biovalue of the fishery at time t.

We begin with the case of an unexploited fishery (Fig. 6). In general, such a fishery will consist of a mixture of several year-classes. The total unharvested biovalue $V(t)$ will be large and relatively constant over time.

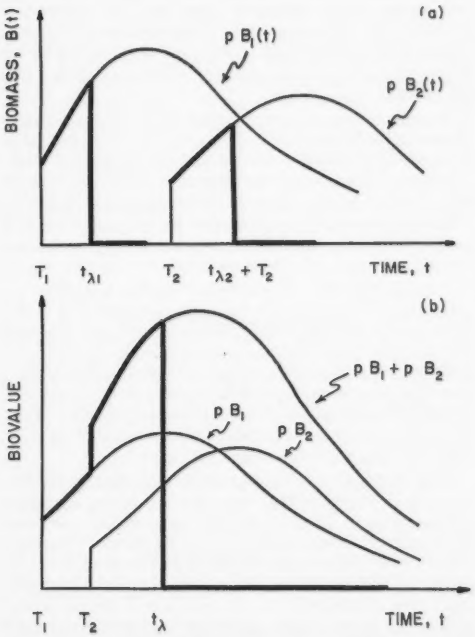

FIG. 4. Optimal fishing; two year-classes: (a) "non-overlapping" case; (b) "overlapping" case.

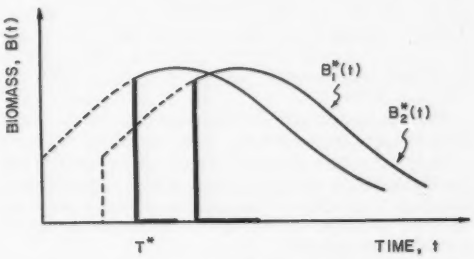

FIG. 5. Mesh-size limitations.

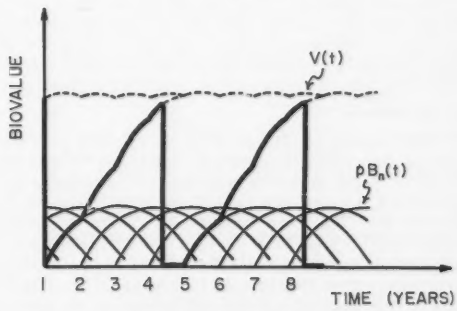

FIG. 6. Optimal fishing — no mesh limitation.

Unless λ is small, the condition $V'/V > \lambda$ may never hold, and will surely not hold throughout the year. Consequently optimal fishing will initially utilize the maximum effort F_{max}. But since fishing reduces all existing year-classes in equal proportion the value

$$\frac{V'(t)}{V(t)} = \frac{\sum B_i(t)\,(w_i'/w_i - M)}{\sum B_i(t)}$$

will not be changed as a result of fishing. Consequently fishing will continue until the entire population has been harvested (or, in practice, until fishing costs become significant and fishing becomes unprofitable).

Because of the assumed constant recruitment rate,[2] however, new year-classes will enter the fishery and the fish population will begin to build up again (Fig. 6). Since the fishery now contains primarily immature fish with large growth rates, the inequality $V'/V > \lambda$ may hold, and may persist for several years. Eventually, however, the average growth rate will decline and the equality $V'(t) = \lambda V(t)$ will occur. The Fisher Rule then requires maximum intensity fishing at this time, again reducing the population to zero and starting the process once more.

Thus with no mesh limitations, the optimal fishing policy may result in a highly unstable fishery, in which, for the case of a long-lived species, several seasons with no fishing are followed by a season with intense fishing. In practice this ideal optimum might be realized by a fishing fleet that alternated its effort between several separate fishing grounds. The technique of periodic, or "pulse," harvesting in order to maximize the average long-term yield from a fishery (the case $\lambda = 0$), has been suggested in simulation studies by Allen (1972) and Walters (1969).

If mesh limitations are taken into consideration, the situation changes remarkably. As in the case of two year-classes, a mesh-size limitation determined by the optimal age t_λ effectively prevents the overlapping of year-classes available to the fishery, and permits separate optimization for each year-class (Fig. 7), thereby attaining the optimum optimorum.

Notice that the optimal mesh-size limitation has the additional effect of stabilizing fishing effort to a constant total seasonal effort. At the beginning of the development stage, however, there occurs (in our idealized situation, at least) an initial season in which a very large fishing effort results in a large harvest, as all the mature fish are removed from the previously unexploited fishery. It

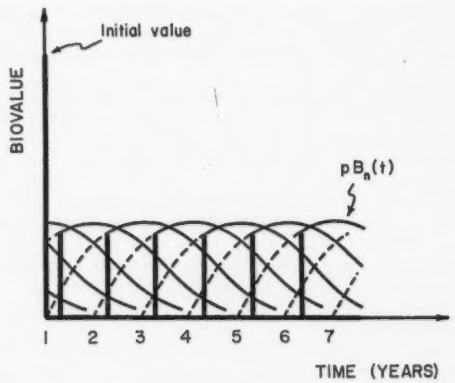

FIG. 7. Optimal mesh-size limitation.

appears to be a characteristic property of virgin renewable resource stocks that an initial "mining" stage is later transformed into a sustained yield situation, with a significantly reduced standing population. This development does not necessarily imply overexploitation. There is, however, a danger that the initial developers, failing to understand the dynamics of the situation, may adopt an unwarranted optimism regarding the long-range productivity of the resource. Other considerations such as stock-recruitment relationships and ecological stability mechanisms may also be involved in the complex problem of overfishing.

Finally let us mention a problem left unanswered by Beverton and Holt (1957, Sec. 19.2.2.), the regulation of an overexploited fishery. From the single year-class model we see that a common-property fishery will always operate at or near the level of vanishing profits. If fishing costs are low, few fish will survive beyond the minimum marketable size. The overexploited fishery will thus consist primarily of immature fish.

Suppose now that a government agency considers the possibility of imposing a mesh-size limitation in order to reduce overfishing and to increase yield. As observed by Beverton and Holt there will necessarily be an initial reduction in fish landings owing to the shortage of mature fish. The greater the mesh-size regulation that is chosen, the more prolonged will be the recovery period. What is the proper trade-off between increased yields and the length of the recovery stage?

This question can be answered immediately on the basis of our theory. The overexploited fishery has been operating as if the discount rate were infinite, whereas the regulated fishery will adopt a finite "social" discount rate λ. The Fisher Rule implies that fish should not be harvested below the optimal age t_λ. Consequently the optimal regulation of the fishery will consist of the immediate adoption of a mesh-size limitation determined by this optimal age. Landings will be significantly reduced (to zero in our idealized model) until the first year-class attains the optimal age, at which time the fishery will

[2]Our conclusion that in a low-cost fishery, optimal fishing will reduce the standing population to zero depends strongly upon the assumption that recruitment and stock size are independent. It is probably fair to assume that for many fisheries the stock–recruitment relation can be virtually ignored, but certainly not for all cases. Fisheries in which recruitment is of dominant importance may be analyzed by means of the logistic model. We hope in a future publication to be able to discuss the case in which growth and recruitment are both of importance.

enter the stage of optimal sustained yield[3] shown in Fig. 7. This yield, and the profits that it generates, will be greater than before, and the increased profits will be sufficiently great to offset the losses incurred during the recovery stage.

We remark again, however, that the optimal sustained yield (for $\lambda > 0$) will be less than the maximum sustainable yield, and the fishery will reach the optimal level sooner than it would reach the level of maximum yield. This is a result of the economic trade-off between yield and length of the recovery stage. This conclusion has been overlooked in most of the conservation literature, and has also been generally misunderstood in the literature on the economics of fisheries (see Clark 1973b, for further details). The greater the discount rate λ, the smaller will the sustained yield be, and in fact (as we have seen in the first section), especially large discount rates may result in sustained yield levels approximately equal to the "rent-dissipating" yields of a common-property fishery.

Since optimal management of a fishery with any given positive discount rate will result in a sustained yield smaller than the maximum sustained yield — and, indeed, smaller than the "eumetric" yield of Beverton and Holt — the question arises of defining the concept of "overfishing." We believe the answer should be given along the following lines. First, there is assumed to exist a socially optimal discount rate λ. Correspondingly there is an optimal sustained economic yield Y_λ, where "economic yield" refers to (average) annual net revenue rather than physical yield by weight.

Overfishing can then be defined as a state in which the existing sustained yield is less than Y_λ, and can be increased only by means of a temporary reduction of yield. Similarly underfishing can be defined as a state in which yields can be permanently increased without a period of temporary reduction. It is clear that, barring "catastrophes of nature," a state of overfishing can be reached only through a process of excessive harvests. A common-property fishery is always overfished according to this definition, but so is any fishery operated for profit, whenever the owners adopt a discount rate greater than the social rate of discount.

A Numerical Example

To exhibit the effect of time-discounting, we consider the North Sea plaice (*Pleuronectes platessa*) fishery considered by Beverton and Holt. The following parameters apply to this species.

Natural mortality rate, $M = 0.10$
Weight curve $w(t) = w_\infty(1-e^{-K(t-\tau)})^3$
where $w_\infty = 2867$ g
$\tau = -0.815$ years
$K = 0.095$

By equation (14) the optimal age t_λ satisfies

$$\frac{w'(t_\lambda)}{w(t_\lambda)} = \frac{3Ke^{-K(t_\lambda-\tau)}}{1-e^{-K(t_\lambda-\tau)}} = M + \lambda,$$

and this has the solution

$$t_\lambda = \tau + \frac{1}{K} \ln\left(1 + \frac{3K}{M+\lambda}\right). \qquad (24)$$

When $\lambda = 0$ we obtain the age of maximum biomass, $t_a = 13.37$ years. If $\lambda = 10\%$, for example, then $t_\lambda = 8.51$ years; the general dependence of t_λ upon λ is indicated in Fig. 8.

The optimal length l_λ can be calculated from t_λ by means of the formula (Beverton and Holt 1957, p. 34 and 285)

$$l_\lambda = L_\infty\,(1-e^{-K(t_\lambda-\tau)}); \qquad L_\infty = 68.5 \text{ cm}$$

We see that the optimal age t_λ is quite sensitive to the values of the instantaneous discount rate λ. This would be expected from equation (24), since the natural mortality rate $M = 10\%$ is of the same order of magnitude as normal discount rates.

How does the sustained yield under optimal fishing compare to the maximum sustainable yield? Let

$$\rho_\lambda = \frac{B(t_\lambda)}{B(t_0)}.$$

If fishing costs are negligible, then most of the biomass will be harvested at age t_λ, so that ρ_λ represents the sustained yield as a proportion of the maximum yield. For $\lambda = 10\%$ we have $\rho_\lambda = 81.4\%$; in general the dependence of ρ_λ upon λ is shown in Fig. 8.

This simple example emphasizes the fact that optimal sustained yield is not the same as maximum sustained yield.

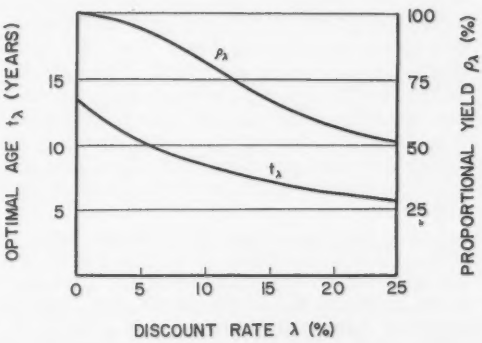

FIG. 8. Optimal age and yield for plaice.

[3]The term "optimal sustained yield" as used here does not involve any stock-recruitment relationship, but refers only to optimization of the size of fish.

Limitation of the Analysis

As an introductory theoretical treatment, the present analysis has been forced to adopt certain unrealistic assumptions, the most serious of which are (a) independence of stock and recruitment, and (b) negligibility of costs. These assumptions have been dictated largely by our desire to present the dynamic aspects of fishery economics in the simplest possible form. Once the basic principle of the Fisher Rule is understood, there is nothing to prevent the inclusion of more realistic assumptions in computer analysis of specific fisheries problems.

If fishing costs are taken into consideration, our theory can still be used to obtain certain results. First, the optimal mesh size will be determined by some age t^* such that

$$t_\lambda < t^* < t_o.$$

Since t^* depends in a complex way upon all the parameters of the problem, and since it appears that t^* will usually be closer to t_λ than to t_o, except for marginally profitable fisheries, it would seem reasonable to choose t_λ itself as an optimal age of harvest. It is possible, however, that the age t_λ would result in a negative biovalue $V(t_\lambda)$, in which case a greater age of entry would clearly be preferable.

The question remains of determining the optimal fishing effort. In this regard, it should be remarked that our analysis has proceded along lines quite different from the analysis of Beverton and Holt, who based their recommendations for controlling fisheries on the concept of "eumetric" fishing (Beverton and Holt 1957; Section 19), which appears to be a purely equilibrium concept.

Beverton and Holt observed, however, that if a mesh-size limitation corresponding to the age t_o of maximum biomass is used, then sustained yield is an increasing function of fishing mortality F (which they assume to be constant over time). The same conclusion may be expected to hold, at least approximately, for the case of the optimal age t_λ, provided that reasonably small discount rates λ are utilized. If so, it follows that physical yields could not be increased by limiting fishing effort. There may still be valid economic reasons, however, for controlling the effort.

Certain fisheries, such as trawl fisheries, may not be amenable to any form of limitation upon the age of first capture. If fishing costs are significant, the theoretical problem of determining the optimal choice of fishing effort in this case seems to be very difficult. To optimize the recovery stage of an overexploited fishery, the best procedure would probably consist of estimating numerically the total present values for a number of alternate choices of regulatory schemes. Examples of the latter are given in Section 19 of the Beverton and Holt work.

Another practical shortcoming of our analysis is its disregard of random variations. Variations in the annual recruitment R should not seriously affect our conclusions; variations in the growth rate of the individual fish would have the effect of smoothing out the theoretically optimal fishing-effort function shown in Fig. 7. Random variations in the economic parameters could also have important implications, but we have not studied this problem.

Finally it must again be emphasized that our study has neglected any possible stock–recruitment relationship, and this could have a serious effect upon the description of an optimal harvest policy, particularly for heavily exploited fish populations. Further research appears to be required to unravel the combined effects of growth and recruitment on optimal fishing policies.

Conclusions

In this article we have discussed a dynamic version of the Beverton-Holt model of a commercial fishery in which stock–recruitment effects can be neglected. We have suggested that optimization in fisheries management would require maximization of the total present value of net revenues. The implications of such a management criterion, in the case of a fishery with negligible costs, include the following:

1. There is an optimal age of harvest t_λ that depends upon the given discount rate λ. If λ is positive then t_λ is less than the age t_o of maximum biomass.

2. If an optimal mesh limitation, determined by the optimal age t_λ, is adopted, then the optimal fishing effort will capture most of the mature fish during each fishing season.

3. The optimal recovery of an overexploited fishery requires an immediate mesh-size limitation as determined by the optimal age of harvest t_λ. The additional revenues from the regulated fishery will be sufficient to cover losses incurred during the recovery stage.

4. If mesh limitations, or other gear limitations affecting the age of harvest, are impractical, then optimization may be achieved through seasonal closures of the fishery. These closures may result in an unstable "pulse" fishery, with large efforts occuring once every several years.

5. Optimal development of an unexploited fishery, particularly of a long-lived species pos-

sessing a large natural population, will result in very large landings during the initial stage of development. These landings cannot be maintained in perpetuity by any regulatory mechanism, however.

6. The physical results of optimal regulation may depend quite strongly upon the value of the given discount rate λ. The use of high discount rates would lead to overfishing, at least in the usual "biological" sense in which sustained yields are significantly lower that maximum sustainable yields.

Mathematical Appendix

In this appendix we prove the complete Fisher Rule under the hypothesis that the expression $V'(t)-\lambda V(t)$ has at most one change of sign. When this hypothesis does not hold, the proof given still suffices to obtain a weak form of the rule. Our proof is elementary and self-contained, and does not rely on variational techniques.

We begin with the single year-class model. The problem then is to maximize the integral

$$PV(F) = \int_0^\infty e^{-\lambda t} F(t)[p\, x_F(t) w(t) - c]dt \qquad (25)$$

where

$$\frac{dx_F}{dt} = -(M + F(t))\, x_F(t), \qquad x_F(0) = R \qquad (26)$$

$$0 \leq F(t) \leq F_{max} \qquad (t \geq 0). \qquad (27)$$

We assume that F_{max} is sufficiently large that the path $B(t)$ determined by the Fisher Rule can be followed.

Henceforth let $F(t)$ denote the effort function specified by the Fisher Rule, and let $F^*(t)$ denote an arbitrary admissible effort function — that is, $F^*(t)$ is a piecewise continuous function ($t>0$) satisfying (27). We will argue by contradiction that $F^*(t)$ is not optimal unless $F^*(t) = F(t)$.

The curve $B_F(t)$ is as indicated in Fig. 2. Suppose for example that the curve $B_{F^*}(t)$ lies below the curve $B_F(t)$ — Fig. 9. If $F^*(t)$ is optimal, we know that $B_{F^*}(t)$ cannot fall below the level $B(t) = c/p$ for $t < t_o$. Hence there exist $T_1 < T_2$ with the property that

$$B_{F^*}(t) < B_F(t), \qquad T_1 < t < T_2;$$
$$B_{F^*}(T_i) = B_F(T_i), \quad i = 1,2.$$

We will establish the following inequality

$$\int_{T_1}^{T_2} e^{-\lambda t} F^*(t)[pB_{F^*}(t) - c]dt < \int_{T_1}^{T_2} e^{-\lambda t} F(t)[pB_F(t) - c]dt, \qquad (28)$$

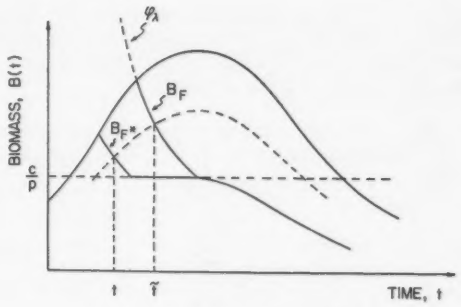

FIG. 9. Proof of the Fisher Rule.

which shows that $F^*(t)$ cannot be an optimal solution, since the principle of optimality (Pontrjagin et al. 1964) states that any subpath of an optimal path must itself be optimal. Inequality (28) shows that $F^*(t)$ is not optimal for $T_1 \leq t \leq T_2$, and hence not optimal overall.

To prove (28) we introduce a transformation $t \longrightarrow \tilde{t}$ (for $T_1 \leq t \leq T_2$) by following the natural biomass curve through the point $(t, B_{F^*}(t))$ until it meets the curve $\phi_\lambda(t)$ at the point $(\tilde{t}, B_F(\tilde{t}))$ — see Fig. 9.

Now the basic equation (26), $dx/dt = -(M + F(t))x$, can be solved explicitly:

$$x_F(t) = x_F(T_1) exp[-M(t-T_1) - \int_{T_1}^{t} F(\tau)d\tau].$$

If $x(t) = x_o(t)$ denotes the natural population curve, then the transformation $t \longrightarrow \tilde{t}$ is determined by the conditions

$$x(t) = e^{-M(\tilde{t}-t)}\, x(t),$$
$$x(t) = x_{F^*}(t); \qquad x(\tilde{t}) = x_F(\tilde{t}).$$

Combining these equations and simplifying, we obtain

$$\int_{T_1}^{t} F^*(\tau)d\tau = \int_{T_1}^{\tilde{t}} F(\tau)d\tau. \qquad (29)$$

By our hypothesis, the "no-fishing" zone is characterized by the inequality $V'(\tau) > \lambda V(\tau)$ where $V(\tau)$ is the natural biovalue; integrating between t and $\tilde{t}$ we conclude that

$$V_F(\tilde{t}) = V(\tilde{t}) > e^{\lambda(\tilde{t}-t)}\, V(t) = e^{\lambda(\tilde{t}-t)}\, V_{F^*}(t). \qquad (30)$$

Inequality (30) has the intuitive interpretation that a unit of fishing effort applied at the point $(\tilde{t}, x_F(\tilde{t}))$ contributes more to present values than a unit of effort applied at $(t, x_{F^*}(t))$. For a "unit of effort" corre-

sponds to $F(t)\Delta t = 1$, and this contribues $e^{-\lambda t} F(t) V(t)\Delta t = e^{-\lambda t} V(t)$ to present values. Inequality (30) asserts simply that $e^{-\lambda \tilde{t}} V(\tilde{t}) > e^{-\lambda t} V(t)$. Since (29) implies that $F^*(t)dt = F(\tilde{t})d\tilde{t}$, we obtain

$$\int_{T_1}^{T_2} e^{-\lambda t} F^*(t) V_{F^*}(t) dt < \int_{T_1}^{T_2} e^{-\lambda \tilde{t}} F^*(t) V_F(\tilde{t}) dt$$
$$= \int_{\tilde{T}_1}^{\tilde{T}_2} e^{-\lambda \tilde{t}} F(\tilde{t}) V_F(\tilde{t}) d\tilde{t}$$
$$= \int_{T_1}^{T_2} e^{-\lambda \tilde{t}} F(\tilde{t}) V_F(\tilde{t}) d\tilde{t}$$

(since $F(\tilde{t}) = 0$ for $T_1 < \tilde{t} < \tilde{T}_1$) and this proves (28).

A similar argument can be used in case $B_{F^*}(t) > B_F(t)$, and we therefore conclude that $F(t)$ must be optimal.

For the case of a population con isting of several year-classes $x_1(t)$, $x_2(t)$, ..., let

$$x(t) = \sum_i x_i(t).$$

Then $\frac{dx}{dt} = -(M + F(t))x$, and the foregoing argument can be invoked verbatim to show that $F(t) = 0$ whenever $V'(t) > \lambda V(t)$. Ambiguous cases arise, however, when $V'(t) < \lambda V(t)$ if this inequality can subsequently reverse direction. It follows from the Pontrjagin maximum principle that either $F(t) = 0$ or $F(t) = F_{max}$ except when $V'(t) = \lambda V(t)$, but we shall not pursue the analysis.

Recently completed computer studies (unpublished) of the North Atlantic cod fisheries by Mr R. Hannesson of the University of B. C. Economics Department have verified and extended the analytical results presented in this paper. Hannesson's model allows for stock-sensitive recruitment relationships, imperfect gear selectivity, and non-negligible costs.

Acknowledgments

We wish to express our gratitude to Professor P. Larkin and to Mr S. Holt for their valuable suggestions. The research has been supported in part by the National Research Council of Canada, grant number A-3990.

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Microcultures of Brown Bullhead (*Ictalurus nebulosus*) Cells: Their Use in Quantitation of Channel Catfish (*Ictalurus punctatus*) Virus and Antibody

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GRATZEK, J. B., M. H. MCGLAMERY, D. L. DAWE, AND T. SCOTT. 1973. Microcultures of brown bullhead (*Ictalurus nebulosus*) cells: their use in quantitation of channel catfish (*Ictalurus punctatus*) virus and antibody. J. Fish. Res. Board Can. 30: 1641–1645.

A microculture system was applied to the culture of brown bullhead (*Ictalurus nebulosus*) cells and used for the titration of channel catfish (*Ictalurus punctatus*) virus and corresponding antibody. As few as 1.7×10^4 cells/well could be used for routine titrations. Simultaneous inoculation of virus with cells at the time of planting resulted in clearer endpoints as opposed to inoculation of preformed monolayers. When channel catfish virus antibodies were measured using plaque reduction and microculture titration assays as indicator systems, no significant differences in antibody titers were noted.

GRATZEK, J. B., M. H. MCGLAMERY, D. L. DAWE, AND T. SCOTT. 1973. Microcultures of brown bullhead (*Ictalurus nebulosus*) cells: their use in quantitation of channel catfish (*Ictalurus punctatus*) virus and antibody. J. Fish. Res. Board Can. 30: 1641–1645.

L'application d'une méthode de microculture à la culture de cellules de barbotte brune (*Ictalurus nebulosus*) nous a permis de titrer le virus de cette espèce et ses anticorps correspondants. Des dilutions aussi faibles que 1.7×10^4 cellules/puits sont suffisantes pour les titrages de routine. L'inoculation simultanée de virus et de cellules au moment de la mise en oeuvre des cultures cellulaires donne des points de virage plus clairs que ceux obtenus avec inoculation de couches monocellulaires préformées. Lorsqu'on utilise la réduction en plaques et le titrage en microculture comme indicateurs pour mesurer les anticorps du virus de la barbotte brune, on n'observe pas de différences significatives dans les titres d'anticorps.

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CHANNEL catfish (*Ictalurus punctatus*) virus (CCV) is a herpesvirus (Wolf and Darlington 1971) first isolated by Fijan (Fijan 1968), which is highly pathogenic for channel catfish fingerlings. The virus replicates in brown bullhead (BB) (*Ictalurus nebulosus*) cells, inducing a cytopathic effect characterized by syncytium formation. The virus will produce plaques when CCV infected BB monolayers are overlaid with agar (Wolf and Darlington 1971). Quantitation of viral neutralizing antibodies can be done using plaque reduction or reduction of cytopathic effects as indicator systems. A need for a microculture system for antibody quantitation developed when it became necessary to measure antibody titers on large numbers of fingerlings from which small quantities of serum could be recovered from one bleeding. Microcultures have been successfully used for detection of various mammalian viruses and corresponding antibodies (Helmke et al. 1970; Schmidt et al. 1966; Sever 1962). These methods in general have proven to be as sensitive, less expensive, and faster than assays utilizing tube cultures.

The objective of this study was to determine the usefulness of BB cell microcultures for viral titration and serological procedures. Experiments were designed to determine the optimal number of cells per culture, the optimum incubation time for virus titrations, the sensitivity of the system in comparison to plaque assays, and its usefulness in serological studies.

Materials and Methods

CELL CULTURES

A starter culture of brown bullhead cells was provided by Dr J. Plumb, Auburn University, Ala. Brown bullhead cells, initially originated by R. G. Malsberger and C. P.

Printed in Canada (J2655)

Cerini at Lehigh University, Bethlehem, Pa. are a continuous line initiated from the caudal trunk tissues of the brown bullhead (*I. nebulosus*). When received, the starter culture had been passed 98 times.

Cell cultures of BB cells were routinely propagated in 75-cm^2 plastic flasks using autoclavable Eagle,s medium supplemented with 15% fetal calf serum (MEM-15) and adjusted to *p*H 7.2 with 3.5% $NaHCO_3$. Penicillin and streptomycin were added to each medium to give a final concentration of 100 units/ml and 100 mcg/ml, respectively. Monolayers were dispersed by standard trypsinization methods (Merchant et al. 1965) and cells were routinely expanded at a 1:3 culture surface ratio and incubated at 29 C.

Cell cultures of BB cells eventually destined for use in plaque assays were established in 60-mm plastic dishes. In this case, BB cells propagated in 75-cm^2 flasks were expanded 1:2. Medium for cells propagated in dishes was MEM-15 buffered with 8.3 mM of $NaHCO_3$ and 20 mM of tris (hydroxymethyl) aminomethane. The final *p*H of the medium was 7.2.

Microcultures of BB cells were established in polystyrene 3.5-cm by 12.5-cm plates containing 96 cups. Medium used was the same as that for dish cultures. Appropriate amounts of cells were distributed into cups using a vertically mounted automatic syringe set to deliver 0.15 ml. Cultures were sealed with tape or polystyrene covers and incubated at 29 C for a period of 3–6 days.

Virus Strain

The Auburn strain of CCV was passed four times in BB cells prior to preparation of working stocks. Virus stocks were prepared by inoculation of BB monolayers with 10^3 plaque forming units (PFU) of CCV. After 24 hr of incubation at 29 C, when 90% of the cells had cytopathic effects, culture fluids were removed, clarified by centrifugation (4 C) at 10,000 × G for 20 min, distributed into 1-ml aliquots and stored at –70 C.

Virus Titrations

Quantitation of virus by plaque assay was based on the method reported by (Wolf and Darlington 1971). Confluent monolayers of BB cells in dishes were inoculated with 0.2-ml aliquots of virus dilutions and the virus allowed to absorb for 1 hr at 29 C. Overlay medium was identical to that used for dish cultures except for the addition of 1.5% agarose. Two milliliters of the overlay medium was added after virus adsorption; the agar was allowed to harden, at which time an additional 3 ml of liquid tris buffered MEM-15 was added. Dishes were incubated in humidified refrigerator-type plastic chambers at 29 C for 48 hr, at which time plaques were made visible by staining monolayers with 3 ml of a 1:5000 aqueous solution of neutral red. Plaque forming unit titers were calculated by multiplying the average number of plaques on two or three replicate cultures times the reciprocal of the virus dilution.

Quantitation by 50% endpoint determination was done utilizing microcultures of BB cells as described above. Aliquots of virus dilutions (.05 ml) were inoculated in microculture cups either prior to seeding with cells or after cell monolayers had formed. When required, quantitation of BB cells was done using a hemocytometer. Microcultures were sealed with tape or covered and incubated at 29 C for 3–6 days. Visualization of cytophathic effects was aided by removal of medium, fixing cells with 10% formalin, staining for 5 min with a 1% aqueous solution of crystal violet followed by a water rinse. Cups without cells appeared clear and were scored as positive; cups with partially destroyed monolayers appeared mottled and were scored as positive. Uninfected monolayers stained blue and were scored as negatives. Fifty percent endpoints were calculated by the Spearman-Karber method (Lennette 1969).

Comparison of Virus Titers Obtained by Inoculation of Preformed BB Monolayered Microcultures with Titers Obtained by Simultaneous Inoculation of Virus and Cells

To determine if inoculation of preformed monolayers was superior to the simultaneous inoculation of virus and cells, six titrations were done comparing methods. Monolayers were produced by seeding with approximately 5 × 10^4 cells/cup and were inoculated with virus 4 days after seeding. Cups receiving virus dilutions and cells simultaneously were planted with approximately 5 × 10^4 cells. Four replicate microcultures were inoculated with each virus dilution. All cultures were incubated for 5 days.

Comparison of Titers Obtained for CCV Inoculated Simultaneously with BB Cells Using Various Cell Concentrations

To establish the optimal number of cells to seed for CCV titrations when virus and cells were inoculated simultaneously, the following experiment was done. Tenfold dilutions of one stock of CCV were made and 0.05-ml aliquots of each dilution were inoculated in each of eight cups of six microtiter plates. A varying amount of cells were delivered to each microtiter plate ranging from a high of 1.4 × 10^5 cells/cup to 1.7 × 10^3 cells/cup.

Comparison of Titers Obtained for CCV Inoculated Simultaneously with BB Cells: Effect of Varying Incubation Times

To determine the effect of the length of incubation time on virus titration results, tenfold dilutions of one stock of CCV were made and aliquots of dilutions inoculated into eight replicate cups in each of five microtiter plates. Approximately 5 × 10^4 BB cells were then added to each cup. After sealing and covering, plates were incubated for 3, 4, 5, 6, and 7 days.

Comparison of Microtiter CCV Titrations with Plaque Titrations

CCV titrations were compared using microcultures and plaque assays. Virus dilutions of six different CCV

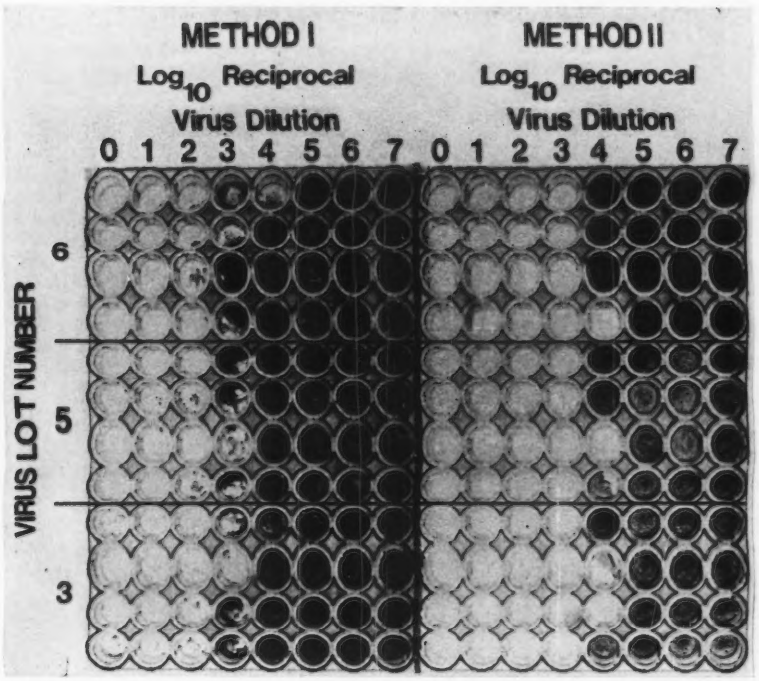

FIG. 1. Comparison of titration methods of channel catfish (*Ictalurus nebulosus*) virus using microcultures of brown bullhead (*Ictalurus punctatus*) cells. Method 1 consisted of inoculation of preformed monolayers; method 2 consisted of inoculation of virus dilution when cells were added to wells.

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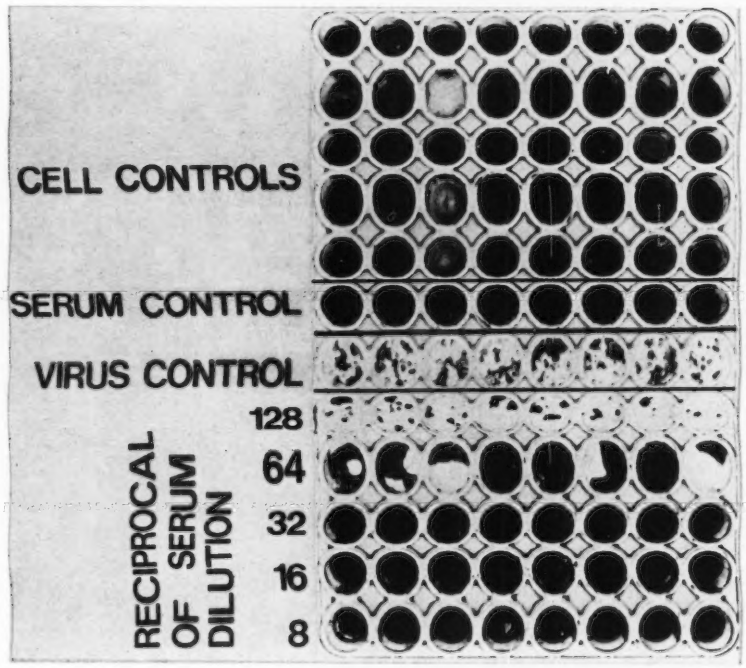

FIG. 2. Virus neutralization pattern using twofold dilutions of catfish serum against 500 PFU of channel catfish virus. Cultures were stained 3 days after titration was initiated.

lots were made and 25-ml aliquots of each were inoculated into 12 replicate microculture cups; 0.2-ml aliquots were inoculated onto petri dish monolayer cultures for plaque assay. Plaques were counted after 48 hr and microculture titration assays were estimated after 3 days incubation.

Virus Neutralization: Comparison of Plaque Reduction Method with Microculture Method

Virus neutralization titers of serums from one naturally exposed and seven hyperimmunized fish were compared using both the plaque reduction and the microtiter sytem sas the indtcator systems. All serums were inactivated by heating at 56 C for 30 min. Serums were diluted in MEM through twofold dilutions to 1:256 starting with a 1:8 dilution of serum. To 1 vol of each serum dilution was added 1 vol of virus containing approximately 500–1000 PFU of virus.

After incubation at 20 C for 1 hr, assay for unneutralized virus was conducted in two ways. In the first method, 0.2-ml aliquots from each serum dilution–virus mixture were inoculated onto each of three replicate brown bullhead cell monolayers. Plaque analysis was done as described above and virus neutralization values for each serum was expressed as that dilution of serum which reduced plaque counts by 50% as determined by the Spearman-Karber method (Lennette 1969).

A second method consisted of inoculation of 0.05-ml aliquots of each serum dilution–virus mixture into microculture cups (eight replicate cups per dilution) prior to seeding each cup with approximately 5×10^4 cells/cup. Endpoint determinations were carried out as described above and the 50% virus neutralization titer of the serum was determined by the Spearman-Karber method (Lennett 1969).

Results

Comparison of Virus Titers Obtained by Inoculation of Preformed BB Monolayer Microcultures with Titers Obtained by Simultaneous Inoculation of Virus and Cells

Five of six titration comparisons resulted in titers within 1 log difference. In one case there was a 1.2 log difference. Endpoint determinations in plates inoculated with cells and virus simultaneously generally resulted in complete destruction of cells as compared to inoculated monolayers as illustrated in Fig. 1.

Comparison of Titers Obtained for CCV Inoculated Simultaneously with BB Cells Using Various Cell Concentrations

Data presented in Table 1 suggests that no significant differences in virus titers were evident when cells were seeded with a minimum of approximately 1.7×10^4 cells/well and a maximum of approximately 14×10^4 cells/well.

Table 1. Titration of channel catfish (*Ictalurus punctatus*) virus by simultaneous inoculation of cells and virus: effect of varying cell concentrations seeded.

Mean no. cells/well[a]	Mean titer[b] log LD_{50}/ml
140,000	5.9(3)[b]
77,000	5.6(2)
38,000	5.3(2)
28,000	5.7(3)
23,000	5.8(3)
17,000	5.9(3)

[a]Mean cell count of three determinations.

[b]Titers indicated are averages of the number of replicate titrations indicated by the number in parentheses. Titers were calculated after a 5-day incubation period. Titers are expressed as the reciprocal of the log dilution of virus which resulted in destruction of 50% of microcultures as estimated by the Spearman-Karber method.

Comparison of Titers Obtained for CCV Inoculated Simultaneously with BB Cells: Effect of Varying Incubation Times

The 50% tissue culture infective dose titer recorded at 3 days incubation, $10^{7.7}$/ml, was not significantly different from the titer of $10^{7.6}$ recorded on day 7. Titers recorded on incubation days 4, 5, and 6 were $10^{7.9}$, and $10^{7.6}$, and $10^{7.6}$, respectively.

Comparison of Microtiter CCV Titrations with Plaque Titrations

Results presented in Table 2 indicate that plaque titers were consistently higher than 50% infectivity endpoints using the microtiter system and averaged about 1.9 times greater on the six titrations.

Virus Neutralization Studies: Comparison of Plaque Reduction Method with Microculture Method

Results of this comparison are presented in Table 3. For all comparisons, serum neutralization titers differed by a factor of 2.2 or less. Figure 2 illustrates a typical microculture virus neutralization pattern.

Discussion

Microculture methods for titration of CCV and CCV antibodies compare favorably with the plaque assay of virus and also as an indicator system for virus neutralization tests. The microculture system has some advantages over other virus titration systems. Since it is possible to seed culture plates at the time of virus inoculation, the 3-day waiting period during which monolayers form can be avoided. In the plaque method, BB cell monolayers form in 3–4 days with an additional 2 days being required for plaque development; in contrast, starting with a "seed" flask of cells, microtiter assay can be completed 3–4 days sooner than the plaque assay. The microtiter assay can be done without the aid of a microscope, thus removing any subjective interpretations of what is or what is not a cytopathic effect. Another important consideration is the efficiency inherent in use of the microculture method for virus neutralization tests. Since one 75-cm^2 seed flask of BB cells can be expanded to produce 10 60-mm petri dish cultures or approximately six microculture plates (576 cups), it follows that by using the microculture technique approximately 12 virus neutralization assays could be done for every plaque reduction assay using a series of twofold serum dilutions. This difference in efficiency is especially significant in view of the comparison of virus neutralization results using both methods. In no instance was there a difference in serum titer greater than a factor of 2.2, and since the minimum significant difference in antibody titer is considered to be fourfold (Hoskins 1957), we conclude that the microculture method is as accurate as the plaque method.

Comparative titrations between various lots of virus using plaque assay and microculture assay resulted in plaque titers being approximately 1.9 times higher than titers obtained by microculture and expressed in ID_{50}/ml. Since it has been established that in systems of equal sensitivity, titers expressed in terms of ID_{50} will be 1.44 times higher than those expressed as PFU/ml (Cooper 1961), we conclude that in the CCV–BB cell assay systems, plaque assay is a more sensitive system than the microculture method.

Under everyday laboratory working conditions, use of BB microcultures for virus neutralization tests, and for routine virus titrations of known lots of CCV, has proven to be a rapid, inexpensive, efficient, and accurate method with which to assay channel catfish virus antibodies.

TABLE 2. Titration of channel catfish virus: comparison of microculture method with plaque method.

Virus lot	Microtiter method ($TCID_{50}$/ml)[a]	Plaque method (PFU/ml)	Titer difference[b] factor
1	7×10^6	1.3×10^7	1.8
2	5×10^6	1.3×10^7	2.6
3	5×10^6	1.5×10^7	3.0
4	9×10^6	1.6×10^7	1.7
5	6×10^6	1.0×10^7	1.5
6	3×10^5	3.5×10^5	1.1
		Avg	1.9

[a]Titers are expressed as the reciprocal of the log dilution of virus which resulted in destruction of 50% of microcultures as estimated by the Spearman-Karber method.

[b]Established by dividing higher titer by lower titer.

TABLE 3. Comparison of virus neutralizing antibody titers for channel catfish virus as determined by plaque reduction and microculture assay.

Serum no.	Virus neutralizing titers[a] as determined by		Titer difference factor[b]
	Microtiter	Plaque reduction	
1	31	33	1.1
2	58	109	1.8
3	22	48	2.2
4	2000	2000	1.0
5	57	83	1.5
6	21	36	1.7
7	21	41	2.0
8	22	42	2.0

[a]Titers are expressed as the reciprocal of that dilution of serum which neutralized 50% of viral infectivity as determined by the Spearman-Karber method.

[b]Titer difference factor is the coefficient of the highest titer divided by the lowest titer.

Acknowledgments

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Digestive Enzyme Secretion After Dietary Variation in the American Lobster (*Homarus americanus*)

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HOYLE, R. J. 1973. Digestive enzyme secretion after dietary variation in the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 30: 1647–1653.

American lobsters, *Homarus americanus*, were fed diets containing 0, 5, and 20% starch for periods up to 37 days. Digestive enzymes in the stomach juices of these and fasted lobsters were assayed. The base-line, or true fasting enzyme level was considered to be those values found after feeding for 2 days on a 5% starch diet. Compared to this basal level both specific activities and absolute amounts of proteinase, lipase and, to a lesser extent, amylase were found to rise on prolonged feeding. Further feeding (all diets) allowed products of a greater proportion of proteinase than amylase or lipase; alteration of the starch content of the diet had no consistent effect.

HOYLE, R. J. 1973. Digestive enzyme secretion after dietary variation in the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 30: 1647–1653.

Nous avons soumis des homards, *Homarus americanus*, à des régimes alimentaires contenant 0, 5 et 20% d'amidon durant des périodes allant jusqu'à 37 jours. Les enzymes digestifs des sucs gastriques de ceux-ci et de homards privés de nourriture ont été analysés biologiquement. Le niveau de base ou vrai niveau enzymatique de jeûne a été défini comme étant les valeurs observées après alimentation de deux jours à un régime de 5% d'amidon. En comparant avec ce niveau de base, on se rend compte que les activités spécifiques, aussi bien que les quantités absolues de protéinase, de lipase et, à un degré moindre, d'amylase augmentent après une alimentation prolongée. Une alimentation plus poussée (à tous les régimes) donne des produits contenant une plus forte proportion de protéinase que d'amylase ou de lipase; les changements dans le contenu d'amidon du régime n'ont pas d'effet uniforme.

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THE phenomenon of adaptation of the digestive mechanism to major dietary constituents has been discussed since Pavlov's time. One type of response, namely a purposeful adaptation, has ample evidence in both vertebrates (Howard and Yudkin 1963; Grossman et al. 1943) and invertebrates (Fisk and Shambaugh 1952; Ishaaya et al. 1971). Grossman et al. (1943) found a pronounced increase in the amylase of rats after feeding a high carbohydrate diet for 3 weeks. Similarly, a high protein diet resulted in an elevated trypsin level. Howard and Yudkin (1963) obtained essentially the same result, but also noted changes in enzyme content after only 1 day's change in diet. Fisk and Shambaugh (1952) have noted a differential stimulation of enzymes dependent on food intake in *Aedes* mosquitoes. Ishaaya et al. (1971) found a strong stimulation of protease activity in cotton worm larvae after increasing dietary protein.

The situation is not always clearly defined, however, and the contrary view, that of parallel secretion, i.e. secretion of a constant proportion of enzymes in the pancreatic juice regardless of diet, is not without support (Babkin 1950; Guth et al. 1956). Guth et al. (1956), using a dog with a permanent pancreatic fistula, measured enzyme concentrations in juice collected after test meals. The variation in enzyme concentration following a test meal was not significantly greater than the daily variation following administration of a standard meal.

Factors which override dietary effects evidently exist. Schlottke (1937) found in the digestive tract of the cockroach (*Periplaneta orientalis*) that proteinase and amylase production was reduced after a high carbohydrate meal. Output of both enzymes was increased after addition of peptone to the diet, but other enzymes, including peptidases, were not affected. Magee and Hong (1956) found

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that the increase in the level of some enzymes was dependent on the presence of certain amino acids in the diet.

In this paper, the effect of variations in the carbohydrate content of the diet on the major digestive enzymes of the lobster is examined.

Materials and Methods

American lobsters, *Homarus americanus*, mixed male and female, were kept in aerated, filtered, flowing sea water, which varied in temperature from 3–10 C. The average weight was 454 g, with a range of 300–600 g. The lobsters were in various stages of the molt cycle, though none had molted within 3 weeks of the experimental period. Food was supplied in 2-g blocks, twice weekly, two pieces for each animal. It was usually ingested within 5 min and always within a half-hour.

Stomach juice was taken 2 days after the last feeding by insertion of plastic tubing through the mouth and withdrawal of all available juice by syringe. Juice was kept cold and centrifuged at 5000 × *g* for 20 min. The supernatant was used for enzyme assay either directly or after a tenfold dilution with water.

The percentage constituents of diets were:

	No starch	Low starch	High starch
Starch	0	5	20
Casein	50	50	50
Gelatin	10	10	10
Vitamins (Castell and Budson 1973)	3.2	3.2	3.2
Minerals (Bernhardt Tomarelli Modified, Nutr. Bioch. Corp.)	3	3	3
Cod liver oil	10	10	10
α-Cellulose	23.8	18.8	3.8

The above mixtures were stirred with 1½ times their weight of nearly boiling water and poured into containers to set, after which they were cut into pieces small enough to be eaten by lobsters and stored at –10 C under nitrogen (Castell and Budson 1973). It will be seen that the proportions of starch and inactive α-cellulose were adjusted to provide diets containing 0, 5, and 20% starch, respectively.

Dietary components were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, 44128.

Enzyme Assays

In the determination of activity optima, assays were carried out from *p*H 2.0–8.5 at intervals of 0.5 *p*H units. Otherwise, tests were made at the *p*H values recorded below.

Amylase — Amylase was measured according to the method of Bernfeld (1955), in citrate–phosphate buffer, *p*H 5.5, at 25 C. Sodium chloride (0.003M) was present as an enzyme activator. One unit of amylase activity is equivalent to 1 μmole of maltose released from amylase per minute.

Proteinase — Protein digesting enzymes were assayed with three substrates: (a) Stomach juice, diluted tenfold, (0.1 ml) was incubated at 37 C with 25 mg Azocoll® (Calbiochem, Los Angeles, Calif. 90054) in 4.9 ml 0.02 M phosphate buffer, *p*H 7.5, or citrate–phosphate buffer, *p*H 5.5. After 10 min vigorous and continuous agitation, the incubation mixture was filtered and the optical density of the filtrate read at 520 mμ against a water blank. Incubation of a buffered Azocoll suspension for 10 min without enzyme produced no detectable absorption; (b) Samples of stomach juice were incubated with 1% casein in 0.02 M phosphate, citrate–phosphate, or citrate buffer. The reaction was terminated by addition of an equal volume of 10% trichloracetic acid. After not less than 30 min standing, samples were filtered and the optical density of the filtrate read at 280 mμ; (c) Hemoglobin (1.5%) was also used as a substrate (Anson 1938). In this case the assay was conducted as with casein. The hemoglobin solution was, in general, made up as follows. Hemoglobin (3.0 g) and urea (36.0 g) were mixed in a mortar. Water (50 ml) and 1.0 N NaOH (8 ml) were added. After stirring at room temperature for 1 hr, the *p*H was adjusted to that required, the solution made up to 100 ml and centrifuged. The supernatant solution was diluted with an equal volume of appropriate buffer before use. Below *p*H 4.5, addition of urea was not necessary for the solubilization of hemoglobin (Wojtowicz and Odense 1970). In this case, the assay itself was not affected.

Lipase — Continuous titration at *p*H 6.5 of fatty acids produced from tributyrin degradation (Brockerhoff et al. 1970) was used. Undiluted stomach juice (0.01 ml) was added to a well-stirred suspension of 0.5 ml tributyrin in 14.0 ml 0.1 M NaCl at 37 C. The rate of addition of 0.02 N NaOH required to maintain *p*H 6.5 was recorded.

Protein — The protein concentration of the stomach juice was measured by diluting 200 times and reading the optical density at 280 and 260 mμ (Layne 1957). The results correlated very well ($r = 0.97$) with those obtained by the Lowry method.

Results

Enzyme Stabilities

The enzyme stabilities shown in Fig. 1 exhibit considerable individual differences. Amylase is fairly stable without freezing, but the proteinases lost activity rapidly, even when frozen. Lipase, although inactivated at 3 C, did not lose its activity rapidly when frozen. Thus, the assay of proteinases immediately after withdrawal of juice from the lobsters' stomachs was necessary. Despite the higher stability of the other enzymes, they were assayed on the day of collection.

*p*H Range of Enzymic Activity

The activities of amylase and proteinase were measured throughout a wide *p*H range, Fig. 2 showing the *p*H-activity profiles. Amylase showed a broad optimum centering at *p*H 5.5. With casein as substrate, proteinase showed two optima at *p*H 2.5 and 5.5. Two *p*H optima (5.5 and 7.5) were also found when Azocoll was used, whereas with hemoglobin as substrate, one optimum was seen at *p*H 4.0. However, in this case the activity extended into the *p*H 7–8 range at about half-optimal value.

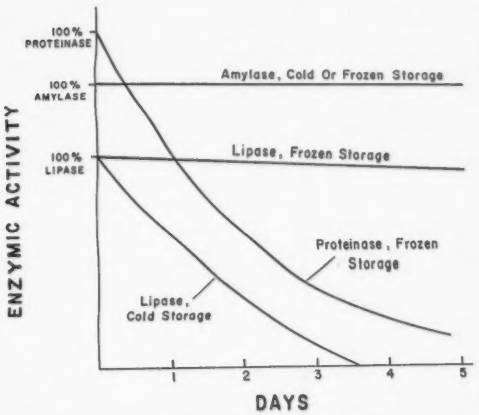

FIG. 1. Stability of lobster stomach juice enzymes during storage. Cold storage was at 3 C; frozen storage at –10 C.

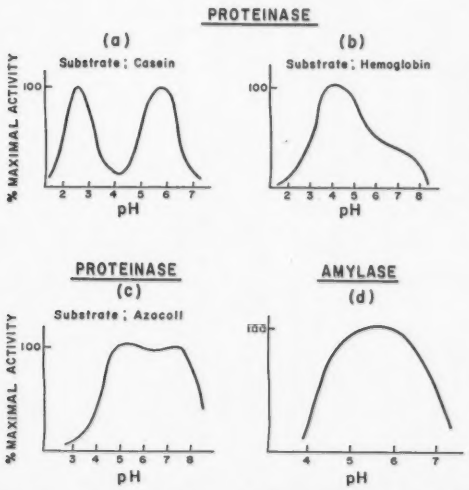

FIG. 2. *p*H activity curves of proteinase and amylase from lobster stomach juice.

Feeding Experiments

Feeding experiments commenced with lobsters which had been fasted for several weeks; the enzyme activities and protein values shown for day zero (Tables 1, 2) are those of unfed lobsters. Feeding was commenced after the first stomach juice samples were taken.

Protein levels in the stomach juice are shown in Table 1. Figures shown represent average values from groups of 4–15 lobsters, with standard error of the mean. Analysis of variance (Pastricha 1969) shows where significant differences between groups exist ($\alpha = 0.05$). The higher concentration in the juices of animals feeding on the 20% starch diet is paralleled by a generally lower gastric juice volume (measured after centrifugation), 1.4–4.6 ml, as opposed to 2.7–6.9 ml for the 0% starch group and 2.1–7.6 ml (with one animal giving 9.2 ml) for the group on 5% starch.

There appears to be little pattern in the variation of total enzyme in the juice with respect to the diets tested (Table 1) except that to some extent fed animals seem to produce more activity. The levels of proteinase at *p*H 7.5 are not tabulated as they are similar to those determined at *p*H 5.5; the ratio proteinase at *p*H 5.5:proteinase *p*H 7.5 varied between the limits 0.7–1.3.

The specific activities of proteinase and lipase were generally higher in well-fed lobsters (Table 2). The ratio of proteinase at *p*H 5.5 to amylase and lipase are also shown in Table 2.

In the absence of starch from the diet, and possibly after prolonged feeding at the low level of 5%, the proteinase *p*H 5.5: amylase ratio is significantly higher than in fasted animals. At the 20% starch level there is less relative difference between the two enzyme levels. No constant relationship occurs in the variation of the proteinase:lipase ratio.

Discussion

The proteolytic activity of the fresh stomach juice with Azocoll as substrate was found to have optima at *p*H 5.5 and 7.5. Although the shallowness of the trough between the peaks of activity (Fig. 2) suggests a broad optimum rather than discrete optima, it is thought that the peaks may correspond to those found by Brockerhoff et al. (1970) with a slightly refined preparation of lobster gastric juice. The optima found here are slightly different from the optima of *p*H 4 and 8 reported

TABLE 1. Protein and enzymes in lobster stomach juice before and after feeding different starch diets. Values shown are the average for the group, with standard error of the mean.

Diet	Days on diet	No. of lobsters in group	Avg vol. of stomach juice (*ml*)	Protein				Enzymes (Total per 0.5 ml stomach juice)					
				Concentration		Total		Amylase[a]		Lipase[b]			
				(*mg/ml*)	LSD[d]	(*mg*)	LSD		LSD		LSD	Proteinase *p*H 5.5[c]	LSD
Fasted	0	6	3.0	65 ±6	30	192[e]±40	155	18 ±4	11.9	23 ±7	39	46 ±7	92
0% Starch	16	12	4.2	80 ±7	26	338 ±44	136	22 ±3	10.2	70[e]±9	34	181 ±25	81
	23	12	3.8	54 ±5.5	26	204[e]±23	136	24 ±2	10.2	–	–	–	–
5% Starch	2	5	6.1	64 ±3	-	389 ±60	–	14 ±6	–	13 ±7	–	18 ±7	–
	37	4	4.6	65 ±8	33	296 ±62	172	9 ±3	12.8	32 ±2	43	192[e]±71	102
20% Starch	13	15	2.8	121[e]±9	25	340 ±37	132	25[e]±3	9.9	76[e]±11	33	124[e]±22	78
F ratio, calculated				12.4		3.1		3.1		9.4		7.4	
F critical, at α=0.05				2.4		2.4		2.4		2.6		2.6	

[a]One unit of amylase activity is equal to 1 μmole maltose released from amylase per minute.
[b]One unit of lipase activity is equal to one microequivalent of fatty acid released from tributyrin per minute.
[c]Measured as optical density units developed per minute.
[d]LSD (Least Significant Difference) indicates the minimum difference which must exist between the indicated figure and the corresponding value for the "5% starch-2 day," fasting, basal level, for significance at the 95% probability level. LSD is calculated from: $\text{LSD} = t\sqrt{\text{MSE}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$, where MSE = mean square error, from analysis of variance (Pastricha 1969), t = percentage point at the number of degrees of freedom of MSE and α=0.05, n_1 and n_2 = sizes of groups under comparison (Petersen 1967).
[e]Significant difference exists between sample and 5% starch — 2-day level of same column.

TABLE 2. Specific activities and ratios of enzymes in lobster stomach juice, before and after feeding different starch diets. Values shown are the average for the group, with standard error of the mean.

Diet	Days on diet	No. of lobsters in group	Specific activities (units[a] per mg protein)						Ratios			
			Amylase (×10)	LSD[b]	Lipase	LSD	Proteinase at *p*H 5.5	LSD	Proteinase :Amylase	LSD	Proteinase :Lipase	LSD
Fasted	–	6	1.9[c]±0.2	0.70	1.1 ±0.2	0.94	1.8[c]±0.3	1.41	0.9[c]±0.3	1.36	1.6[c]±0.4	0.49
0% Starch	16	12	1.3 ±0.1	0.61	2.3[c]±0.3	0.83	3.8[c]±0.3	1.23	2.9[c]±0.3	1.20	1.7 ±0.3	1.10
	23	12	2.9[c]±0.2	0.61	–	–	–	–	–	–	–	–
5% Starch	2	5	0.9 ±0.1	–	0.4 ±0.2	–	0.3 ±0.1	–	0.3 ±0.1	–	0.9 ±0.2	–
	37	4	0.6 ±0.2	0.78	1.2 ±0.4	1.4	3.9[c]±3.5	1.56	6.5[c]±1.7	1.51	3.3[c]±1.4	0.61
20% Starch	13	15	1.5[c]±0.1	0.60	2.2[c]±0.2	0.8	2.5[c]±0.3	1.20	1.7[c]±0.2	1.16	1.1 ±0.2	1.14
F ratio (caculated)			17.3		12.8		11.7		18.0		10.2	
F (critical)			2.41		2.62		2.56		2.62		2.62	

[a]See legend to Table 1 for definitions of units of enzyme activity.
[b]For calculation of Least Significant Difference, see legend to Table 1.
[c]Significant difference exists between sample and 5% starch — 2 day level of same column.

by Brockerhoff et al., but the earlier work used freeze-dried juice which had been adjusted to *p*H 8 and dialysed. Hemoglobin did not seem to be a suitable substrate for determining activity at the higher optimum in view of the lack of a definite peak of activity. The apparent optima of *p*H 2.5 and 5.5 found with casein as substrate are possibly spurious since the trough between the peaks coincides with the region of insolubility of casein. Although use of the three substrates shows a broad range of proteolysis from *p*H 2.5–8.5, Azocoll was chosen as the most suitable substrate for a definitive study in view of its discrete activity optima.

The *p*H for optimum activity of amylase reported here (5.5) agrees well with that (5.3) reported by Wojtowicz and Brockerhoff (1972) for purified lobster juice amylase, using soluble starch as substrate. All crustacean amylases, however, do not lie in this range. Optima of *p*H 6.0–6.8 have been reported for *Thalamita crenata* (van Weel et al. 1969).

Whereas the *p*H of stomach juice of well-fed crustaceans is generally higher than that of fasted animals, (van Weel 1970), it was found to be fairly constant for *Homarus americanus*, varying between 4.6 and 4.8 for both fed (all diets) and fasted animals. It is possible that casein, which was high in all diets, was acting as a buffer here.

Increase of enzymes on feeding may be expected for two reasons: 1) supply of the necessary amino acids, 2) induction of enzyme production by specific components, or digestion products of the diet. Contrary to this expectation, the immediate response to feeding seemed to be a generally reduced enzyme production (see the 5% starch — 2-day level). However, it is thought that measurements made on fasted lobsters might be indicative of an accumulation of unused enzymes, suddenly released in response to the diet. Furthermore, since a true adaptation to diet might not be expected within a 2-day period, this lower 2-day level probably represents the true "starvation basal level." Hence, the values obtained after a longer period of feeding have been compared to this lower level.

The basic diet used in the experiments reported here, i.e. with 5% starch, has been shown to promote growth in adult lobsters (Castell and Budson 1973). In developing this diet, Castell and Budson have shown that casein alone, as a granular rather than a reprecipitated or soluble preparation, promotes growth of lobsters. They demonstrated increased serum protein levels, increased tissue growth and an increase in the edible portion of lobsters maintained on a diet containing casein as the sole protein source.

Since the diet used in these experiments contains the major nutrient classes, it might be expected to allow production of substantial quantities of digestive enzymes. This it does in the case of lipase and proteinase both in absolute amount (Table 1) and in specific activity (Table 2). With one anomalous result (5% starch — 37 days), the same is true for amylase.

The exclusion of starch from the diet does not seem to lower the total amylase production (as calculated from data in Table 1), in fact, amylase of high specific activity can apparently be produced under these conditions. This argues against an immediate purposeful adaptation of one of the major digestive enzymes to its substrate. Similarly, the relatively low proteinase: amylase ratio obtained after feeding a 20% starch diet, when compared with prolonged feeding on 0 or 5% starch, does not support a hypothesis of preferential production of amylase. On the other hand, the proteinase:amylase ratio and, to a less significant extent, the proteinase: lipase ratio (Table 2), both show an increase over the 2-day base line level after prolonged feeding. This suggests a preferential production of proteinase and, since proteins comprise 60% of all three diets, a possible adaptation to this substrate. Perhaps this is what would be expected for an animal which thrives on the mixed diet available in its habitat. A low level of production of all major enzymes would allow for immediate digestion of almost any available foodstuff, supplemented by a subsequent increased production of enzymes necessary to deal with a substantial quantity of food material as it becomes available.

Acknowledgments

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First Records of the Gymnoblastic Hydroid, *Ichthyocodium sarcotretis*, on the Copepod, *Sphyrion lumpi*, from Redfish of the Northwest Atlantic

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TEMPLEMAN, W. 1973. First records of the gymnoblastic hydroid, *Ichthyocodium sarcotretis*, on the copepod, *Sphyrion lumpi*, from redfish of the northwest Atlantic. J. Fish. Res. Board Can. 30: 1655–1660.

The gymnoblastic colonial hydroid *Ichthyocodium sarcotretis* was found on the copepod *Sphyrion lumpi* on redfish from three areas of the northwest Atlantic: on copepods (three of ten) on deepwater redfish (*Sebastes mentella*) from about 200–500 m over great depths at the mouth of the Labrador Sea and, in bottom otter trawling, on copepods on *S. mentella* or possibly *S. fasciatus* from the Labrador Shelf (6 of 686) and the northeast Newfoundland Shelf (1 of 364). None were found on 492 *S. lumpi* from redfish taken on the continental shelf south and west of the northeast Newfoundland Shelf.

The characteristics of the hydroid colonies and of their feeding polyps and reproductive hydranths and medusae are compared with published information on this hydroid found on the same hosts in the Irminger Sea and on the copepod, *Sarcotretes scopeli*, on the lantern fish, *Benthosema glaciale*, in the North Atlantic. The incidence of the hydroid on *S. lumpi* on redfish may possibly help in distinguishing *Sebastes* species in the northwest Atlantic.

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L'auteur a trouvé l'hydraire gymnoblastide *Ichthyocodium sarcotretis* sur le copépode *Sphyrion lumpi* présent sur le sébaste atlantique de trois régions de l'Atlantique Nord-Ouest: sur des copépodes (trois sur dix) présents sur des sébastes de profondeur (*Sebastes mentella*) capturés à environ 200–500 m au-dessus des grandes profondeurs à l'entrée de la mer du Labrador et, au cours de chalutages de fond, sur des copépodes présents sur *S. mentella* ou possiblement *S. fasciatus* du plateau labradorien (6 sur 686) et du secteur nord-est du plateau terreneuvien (1 sur 364). Il n'en a trouvé aucun sur 492 *S. lumpi* présents sur des sébastes.

L'auteur compare les caractéristiques de ces colonies d'hydraires, de leurs polypes nourriciers et de leurs hydranthes reproducteurs avec les descriptions de cet hydraire trouvé sur les mêmes hôtes dans la mer Irminger et sur le copépode *Sarcotretes scopeli* présent sur la lanterne glaciaire, *Benthosoma glaciale*, en Atlantique Nord. La présence de l'hydraire sur *S. lumpi* du sébaste peut aider à différencier les espèces du genre *Sebastes* de l'Atlantique Nord-Ouest.

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THIS report records occurrence of the gymnoblastic hydroid, *Ichthyocodium sarcotretis* Jungersen, on the copepod, *Sphyrion lumpi* Krøyer, on redfish (*Sebastes mentella* Travin and possibly *fasciatus* Storer) from the Labrador Sea and the continental shelf of the northwest Atlantic. The hydroid had been found on this copepod on *S. mentella* at Weather Station Alpha in the Irminger Sea by Jones (1966). The only other records of the hydroid were from the copepod, *Sarcotretes scopeli* Jungersen, on the lanternfish, *Benthosema glaciale* (Reinhardt) (=*Scopelus glacialis* Reinhardt), from the northeast Atlantic to off West Greenland (Jungersen 1913a, 1913b; Jones 1966).

Especially as the copepod hosts differ greatly in size, and to compare the colonies with those described by Jones (1966) and by Jungersen (1913a, 1913b), various characteristics of the hydroid colonies, feeding polyps, and reproductive hydranths are described.

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On admittedly slender evidence, the hypothesis is proposed that incidence of *S. lumpi* on redfish will help in distinguishing *Sebastes* species in the northwest Atlantic.

Materials and Methods

The new records (Fig. 1, Table 1) are of 10 hydroid colonies. Three were taken in 1965 (on 3 of 10 *S. lumpi* from *S. mentella*) in fishing for redfish by floating longline at 180–450 m (bottom 3100–3300 m) at the mouth of the Labrador Sea. The rest were taken from 1954 to 1960 at 290–470 m in bottom otter trawling for redfish on the continental slope of the Labrador and northeast Newfoundland shelves (on 7 of 1050 *S. lumpi* from redfish, some called *S. mentella* and others "redfish" when the hosts were recorded).

I noted and preserved the Labrador sea colonies at sea immediately after capture. The colonies are complete and the relative infestation of copepods accurate. The copepods from the continental slope were taken from the redfish by Biological Station technicians and preserved as *S. lumpi*, and examined for hydroid colonies many years later. As some of these colonies appear to be incomplete and portions may have been lost, the incidence of infestation may be minimal.

The copepods with the attached hydroid colonies were preserved in 10% formalin and transferred to 50% isopropyl alcohol before the detailed examination.

Comparison of the colonies with the description of those found by Jones (1966) indicated that they were of the same species. Data on length and number of endodermal ridges of gasterozooids, length of reproductive hydranths, and length of medusa buds in the new specimens and the data available for Jones' were compatible.

Transverse sections of Jones' (1966) gasterozooids had eight endodermal ridges at the oral end which were reduced in size and joined together towards the basal region. In the few western Atlantic polyps sectioned, gasterozooids usually possessed eight endodermal ridges but sometimes less, and up to 11 near the lip of the polyp and increases of as much as from six or eight at the lip to 12 further back in the polyp cup. This is very likely not a difference as there is no indication that Jones investigated the problem in detail.

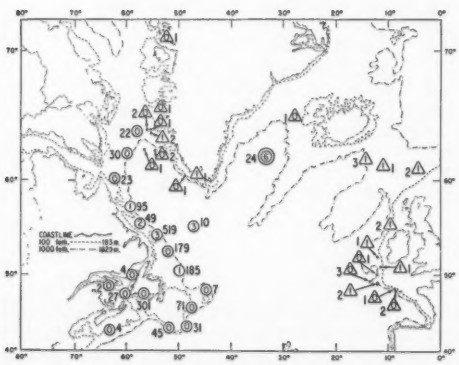

FIG. 1. Known distribution of the colonial hydroid, *Ichthyocodium sarcotretis*, in North Atlantic. Numerals inside circles and triangles, *I. sarcotretis*; outside, copepod hosts examined. Single circles, records from the copepod *Sphyrion lumpi* on redfish from the northwest Atlantic (Table 1); double circles, those of Jones (1966). Triangles, records from Jungersen (1913a, 1913b) and Jones (1966) of *I. sarcotretis* from the copepod, *Sarcotretes scopeli*, on the lanternfish, *Benthosema glaciale*.

Distribution, Form, and Abundance of Hydroid Colony and Relation to Copepod and Fish Hosts

The new records of *I. sarcotretis* extend its known range from West Greenland and, in relation to its presence on *S. lumpi* on redfish, from the Irminger Sea to the midmouth of the Labrador Sea and to the continental shelf off Labrador and northeast Newfoundland (Fig. 1, Table 1).

The hydroid was relatively abundant on *S. lumpi* from pelagic redfish of the Labrador Sea (30% infested), and less abundant but not rare on these copepods from redfish of the Labrador coastal shelf (0.9%) and of the northeast Newfoundland Shelf (0.3%). The hydroid was not found further south although 492 *S. lumpi* were examined, including 301 from Hermitage Bay (Fig. 1, Table 1).

When observed fresh after capture, the hydroid colonies from the Labrador Sea (Table 1: no. 1–3) were reddish, and appeared to be much more diffuse and to cover a greater area than after preservation in formalin (Fig. 2). These were large, mature, and complete colonies, 46–60 mm long, covering a great deal of *S. lumpi* and having in relation to the copepod a clear division into an anterior trophosome of feeding polyps (gasterozooids) beginning from a base around the posterior part of the neck of the copepod, and a posterior gonosome of reproductive hydranths with fairly well-developed medusa buds projecting from them. The colonies from the continental shelf (Table 1: no. 4–10) were smaller. In both groups the gasterozooids were always situated anteriorly on *S. lumpi* and the reproductive hydranths with medusa buds posteriorly. In four out of six of the shelf colonies in good condition (Table 1, specimens 4–6, 9), the colony began anteriorly on *S. lumpi* as a ring around the base of the neck of the copepod. In specimen 7, in which part of the colony was probably lost, both gasterozooids and reproductive hydranths were posteriorly on the trunk but with the gasterozooids, as usual, anterior to the reproductive hydranths. In specimen 8, almost all the colony was lost, only a few gasterozooids remaining. In specimens 4 and 9, the presence

TABLE 1. Records of *Ichthyocodium sarcotretis* on *Sphyrion lumpi* of the northwest Atlantic, 1954–65.

Specimen[a]	Date of capture	Capture location		Depth of capture (*m*)	Bottom temp. (*C*)	Length of hydroid colony (*mm*)	Greatest length (*mm*)			Greatest width, trunk of host (*mm*)
		N	W				Gastero-zooids	Medusa buds[b]	Reproductive hydranths	
1[b]	July 18, 1965	55°46′	47°17′	400–450	3.9–4.0	60	14	1.7	18	11[c]
2[b]	July 18, 1965	55°46′	47°17′	400–450	3.9–4.0	50	9	1.2	13	15[c]
3[b]	July 16, 1965	55°29′	47°08′	180–400	3.6–3.9	46	12	1.5	9	19[d]
4	July 31, 1960	57°00′	59°12′	450–457	4.48	23+	6	0.6	–	16[e]
5	Aug. 4, 1960	55°26′	56°29′	435–468	3.27	–	6	0.9	–	17[e]
6	Aug. 4, 1960	55°26′	56°29′	435–468	3.27	–	8	0	–	14[c]
7	Aug. 5, 1960	54°45′	53°50′	320	4.00	9	4	0.8	3	9[c]
8	Sept. 6, 1954	54°46′	53°53′	291	1.11	–	–	–	–	ca. 22[f]
9	Sept. 16, 1954	54°44′	53°50′	296	1.82	22	8	1.5	6	20[c]
10	Oct. 5, 1959	50°58′	50°38′	448–461	3.81	17	7	1.2	8	9[c]

[a]The redfish hosts for specimens 1–3, *S. mentella*, were caught pelagically in the Labrador Sea over water 3100–3300 m deep. The other redfish, taken in bottom otter trawling on the continental shelf off Labrador and northeast Newfoundland, were recorded at time of capture as *S. mentella* (specimens 4–7) and "redfish" (specimens 8–10).

[b]Tentacles included.

[c]No egg sacs present on *S. lumpi*.

[d]Egg sacs present on *S. lumpi* but abnormal or hatched.

[e]Normal egg sacs present on *S. lumpi*.

[f]*Sphyrion lumpi* and *I. sarcotretis* dried up. Only remnants of base and of a few gasterozooids remaining.

of a basal plate extension of the colony, with no related gasterozooids or reproductive hydranths, suggests loss of a portion of the colony. Some collectors may have cleaned these and other specimens of *S. lumpi*, thus injuring or destroying hydroid colonies.

The gasterozooids varied considerably in shape and size of the polyp cup and in length of stalk (Fig. 3A). They were without tentacles, as is typical of the species and its relatives. More than one polyp often arose from a single stalk (Fig. 3B), and many gasterozooids from a matted group of stalks on a basal plate which acts as a hydrorhiza (Fig. 3D).

Observations were made on numbers of endodermal ridges (Fig. 3C) on 20 polyps sectioned for photography. The usual number just behind and presumably at the lip at the oral end of the polyp was eight, although 6–11 were observed. Sometimes sections further back in the cup of the polyp had the same number of ridges as near the lip but in others the number increased from six or eight to 12. In one polyp investigated throughout, the number increased from eight near the lip to 12–13 further back in the cup and decreased gradually to eight in the neck and to five in the stalk close to the cup. In another polyp split lengthwise, there were 10 ridges near the lip; seven of these continued as single ridges, one divided into two, another divided into three and one tapered off and came to an end so that the number was 12 at $\frac{5}{8}$ of the distance from the lip.

Reproductive hydranths of the hydroid derive from gasterozooids and in typical condition each, even when fully developed, has a feeding polyp at its distal tip. Both gasterozooids and reproductive hydranths at times arose anteriorly and posteriorly, respectively, from the same small portion of the basal disc (Fig. 3D).

The early development of medusa buds on a gasterozooid, and their gradual growth and development into a mature reproductive hydranth with relatively large medusa buds with tentacles, are shown in Fig. 4. Note the feeding polyp at the distal tip of each reproductive hydranth.

The attached medusa buds (Fig. 5) in moderate and later stages of development (Fig. 5) usually had two and occasionally three well-developed tentacles. The tentacles had many different shapes. There were patterns of resemblance between some medusa buds but not all. Some differences between tentacles were presumably due to differences in stage of development, but the variety was great enough to allow the possibility of real differences.

In the Labrador Sea, well-developed medusa buds were found in July; on the continental shelf, in September and October (Table 1).

Three of the 15 redfish taken in July 1965, over oceanic depths in the Labrador Sea, were infested with the copepod. Three of the 10 copepods found had well-developed epizoic colonies of the hydroid. Each of the three redfish had a colony of the hydroid on one copepod (one of one, one of two, and one of seven). The copepods infested were among the larger ones. One copepod had egg sacs with eggs possibly liberated and two moderately large copepods had no egg sacs.

The colonies from the Labrador Sea, taken in mid-July, had only a few medusa buds possibly large enough for release and many smaller, developing buds. In contrast, Jones (1966) assumed that medusae were released at Station Alpha mainly in summer. However, the buds in the colonies from the shelf areas, taken on September 16 and October 5, were similar in condition and size to those from the Labrador Sea, taken in July, and to Jones, Irminger Sea medusae, taken in May and June. The lack of larger medusae buds in some of the shelf specimens in late July and August (Table 1: no. 4–6) may be related to loss of the posterior and best-developed reproductive hydranths. It is also possible that liberation of medusa buds (if it occurs), or at least of sexual products, is later on the Labrador and northeast Newfoundland shelves than over oceanic depths in the Labrador Sea.

But Jones' (1966) and my observations confirm Jungersen's (1913a, 1913b) observations that usually two well-developed tentacles are present on the larger gonophores. Kramp's (1922) assumption that medusa buds (gonophores) remain attached to the hydranths, even when mature, was based on the belief that tentacles were absent from the gonophores.

Jones (1966) concluded that the hydroid on *S. lumpi* from Station Alpha was sufficiently similar to *I. sarcotretis* found on the copepod *S. scopeli* to be considered as the same species. The hydroid on *S. lumpi* covered a surface area five times as great as that on *S. scopeli* and the dactylozooids were three or four times as long, but *S. scopeli* is a much smaller copepod than *S. lumpi*. The hydroid colonies on *S. lumpi* from the Labrador Sea were still larger than those from Station Alpha.

Jones (1966) noted the lack of previous records of an association between *I. sarcotretis* and either *S. lumpi* or *S. mentella* and therefore suggested that *I. sarcotretis* did not occur on this copepod on redfish of the coastal and shelf areas. Until the search of the St. John's collections yielded records of *I. sarcotretis* on *S. lumpi* from redfish of the continental shelf, I should have agreed with him.

The colonies of *S. lumpi* appear to be much more abundant and larger on the pelagic redfish over oceanic depths than on redfish of the neighboring

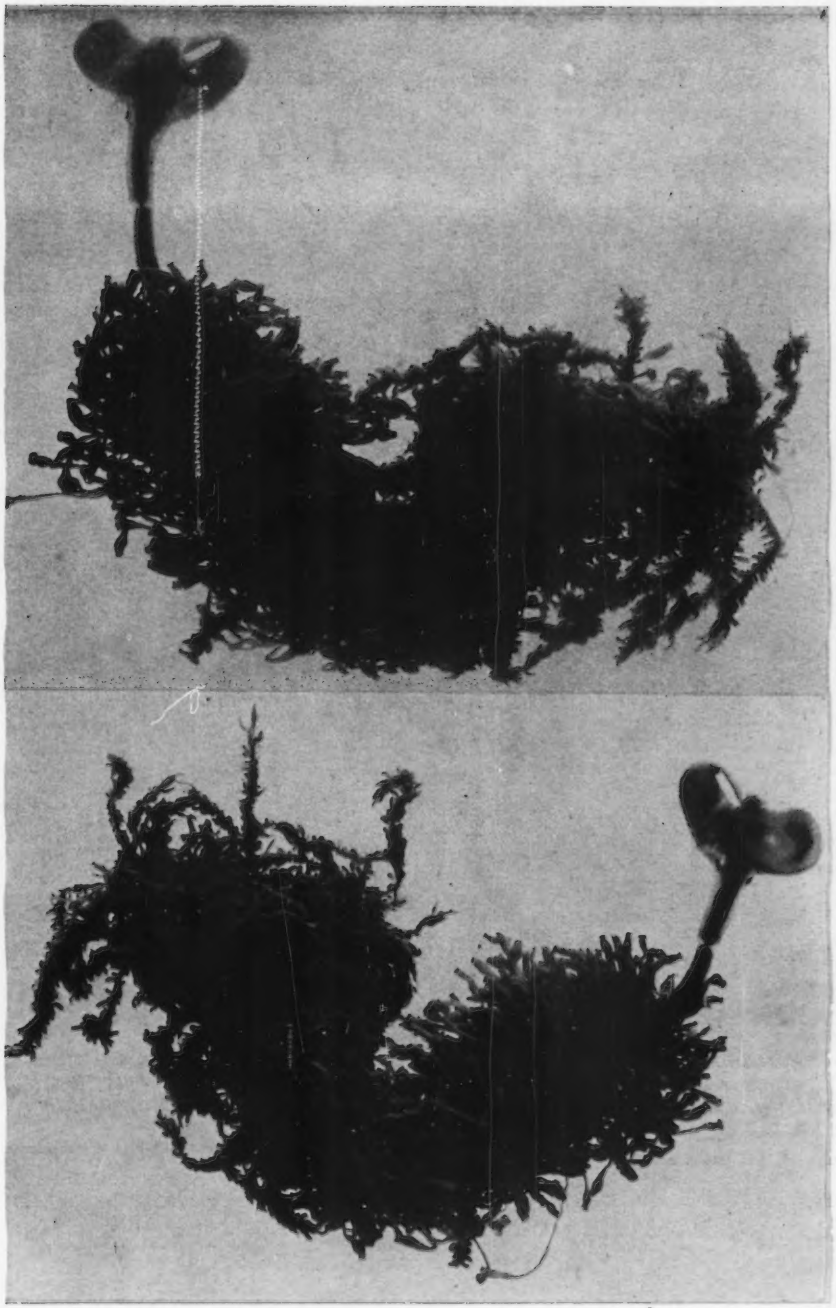

FIG. 2. Ventral (above) and dorsal (below) views of the hydroid, *I. sarcotretis*, on *S. lumpi*. (Specimen no. 1 of Table 1 × 2.5. The "ventral" and "dorsal" refer to *S. lumpi*.)

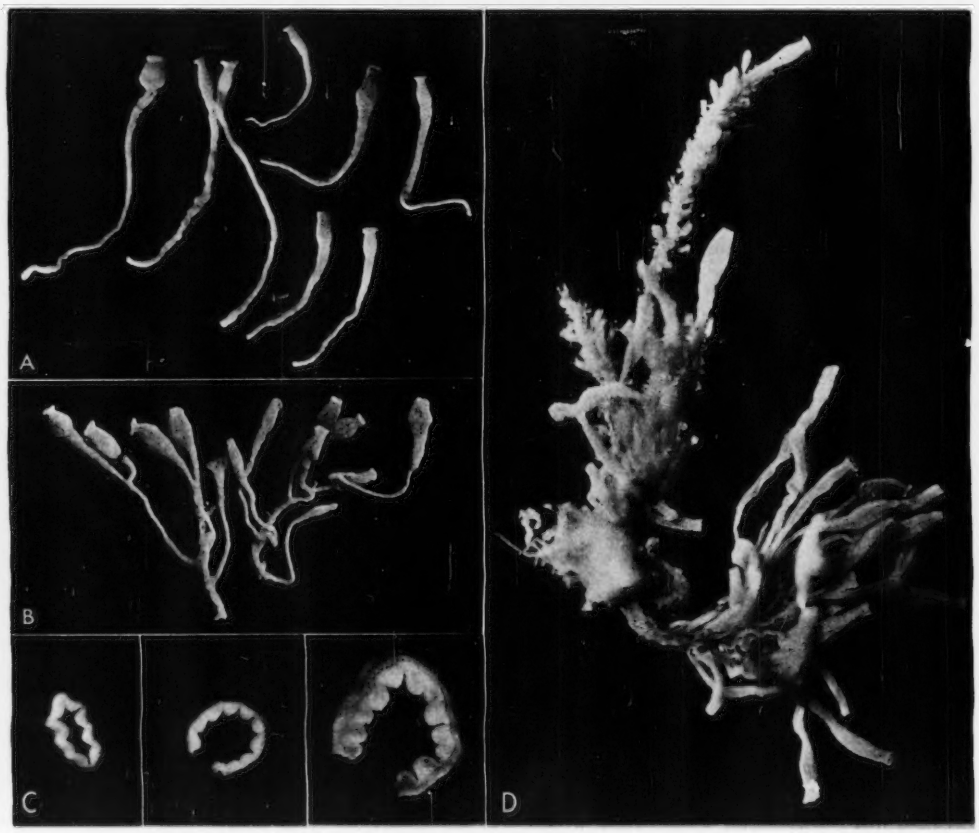

FIG. 3. Portions of *I. sarcotretis* colonies of Table 1: A, gasterozooids of various shapes; B, groups of gasterozooids × 4.7; C, sections × 21 through the cups of gasterozooids showing 8, 10, and 12 endodermal ridges; D, group of intermediate stage reproductive hydranths (left) and of gasterozooids (right) arising from a portion of the basal plate at lower left, × 5.7.

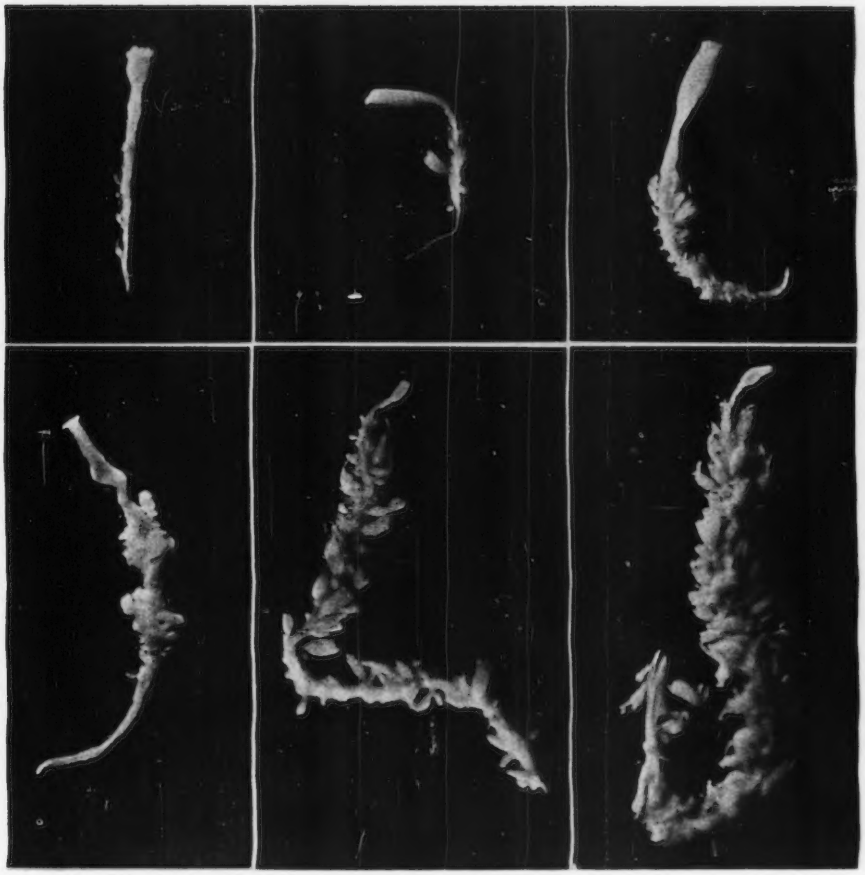

FIG. 4. Gradual development of reproductive hydranth of *I. sarcotretis* from gasterozooid or feeding polyp (from upper left to lower right) × 7.0.

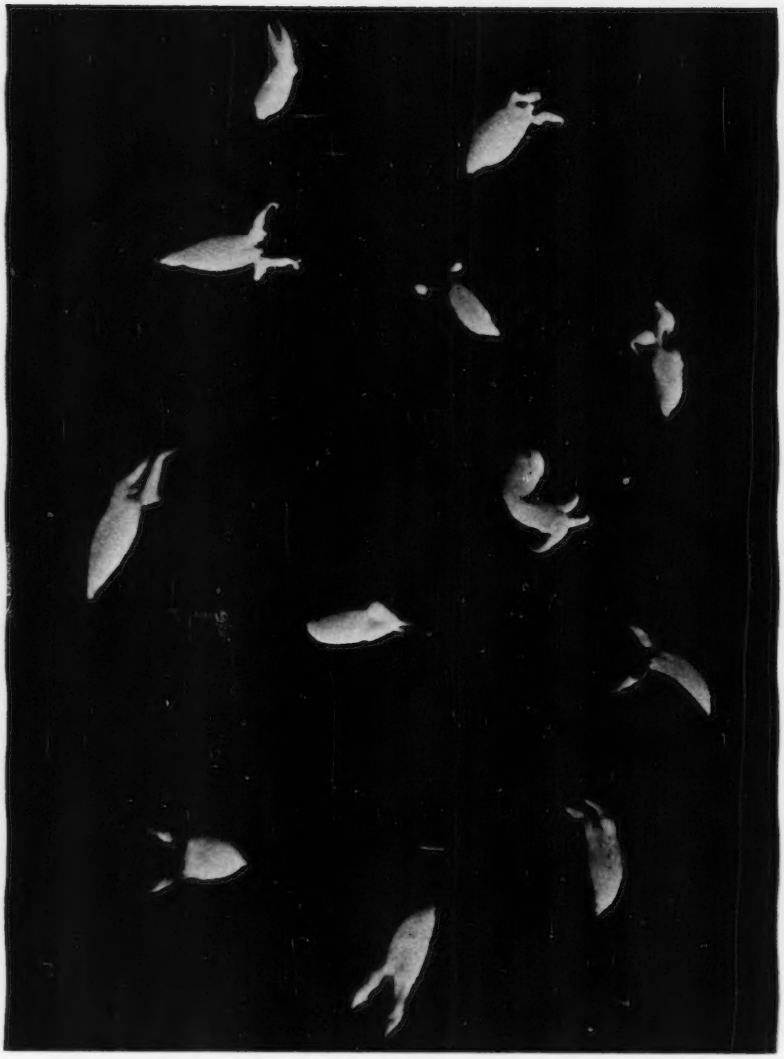

FIG. 5. Medusa buds from *I. sarcotretis* colonies × 22.

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continental shelf. The significance of this cannot be assessed at present because of the possibility of artificial loss of colonies or parts of colonies from the shelf *S. lumpi* before examination.

Apparently the pelagic *S. mentella* of the Labrador Sea are part of the oceanic population found southwest of Iceland and south of Greenland, and similar to European and central North Atlantic *S. mentella* of the northeast Atlantic in having larvae without subcaudal melanophores (e.g. Tåning 1949; Henderson 1961, 1964, 1968; Templeman 1967; Bainbridge and Cooper 1971). This is in contrast to the presence of subcaudal melanophores in unextruded larvae from North American redfish from Hamilton Inlet Bank, Flemish Cap, Hermitage Bay, and the North American shelf (Templeman and Sandeman 1959), also called *S. mentella* by most investigators.

The possible mingling of the two forms of redfish on the shelf is especially of interest in distinguishing *Sebastes* spp. in the northwest Atlantic. Tåning was inclined to use the name *Sebastes fasciatus* Storer 1854, for the North American redfish commonly called *S. mentella* (Tåning 1949; Tåning 1961). Also, Barsukov (1972) and Barsukov and Zakharov (1972) reported data supporting Tåning's usage and indicating that *S. fasciatus*, *S. mentella*, and *S. marinus* intermingle on the northeast Newfoundland Shelf, whereas mainly *S. fasciatus* is found on the southern Grand Bank and only *S. fasciatus* on the Scotian Shelf and Georges Bank.

The lower incidence of *I. sarcotretis* colonies on *S. lumpi* from shelf redfish than on those from oceanic *S. mentella* may be associated with the possibility that most of the shelf redfish bearing the hydroid are of the oceanic population. Of two *S. mentella* females examined by Templeman and Sandeman (1959) from the part of the area where *I. sarcotretis* was found on the shelf population (Hamilton Inlet Bank), both contained larvae with subcaudal melanophores. It is to be expected that the fringe of the pelagic oceanic population of *S. mentella* would mingle to some degree with the population of redfish of the North American shelf.

Redfish on the seaward slope of Hamilton Inlet Bank, where most of the shelf colonies of *I. sarcotretis* were found, are much more heavily infested by *S. lumpi* than redfish are known to be in any other area of the northwest Atlantic except the Gulf of Maine (Templeman and Squires 1960). In the neighboring oceanic area, on the basis of a few pelagic specimens examined (Templeman 1967), redfish also appear to be heavily infested by *S. lumpi*, possibly more heavily than those of the neighboring Labrador Shelf. The oceanic *S. mentella* south of Greenland and Southwest of Iceland, which presumably are of the same population as the redfish over oceanic depths in the Labrador Sea, have similarly high rates of infestation by *S. lumpi* (Zakharov 1964; Jones 1966). At and near Station Alpha, 15% of 267 *S. mentella* were infested by *S. lumpi* and 6.8% of the 88 *S. lumpi* carried *I. sarcotretis* (Jones 1966). These compare with the 20% of 15 *S. mentella* carrying *S. lumpi* and 30% of 10 *S. lumpi* carrying *I. sarcotretis* in my specimens from over oceanic depths in the Labrador Sea.

Acknowledgments

I am grateful to Mr E. J. Sandeman for supervising the preliminary examination of *Sphyrion lumpi* from the continental shelf areas for the presence of *I. sarcotretis*. Photographs are by Mr E. L. Rowe.

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Fatty Acid Content of Coho Salmon, *Oncorhynchus kisutch* — A Statistical Approach to Changes Produced by Diet[1]

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TINSLEY, I. J., H. M. KRUEGER, AND J. B. SADDLER. 1973. Fatty acid content of coho salmon, *Oncorhynchus kisutch* — a statistical approach to changes produced by diet. J. Fish. Res. Board Can. 30: 1661–1666.

Stepwise multiple regression procedures have been used to develop equations expressing the fatty acid content of fingerling coho salmon, *Oncorhynchus kisutch*, as a function of fish length and the time the salmon had been maintained in the laboratory on a specific diet. High correlation coefficients from the multiple regressions were obtained for most of the fatty acids studied and from the derived equations the fatty acid content of individual fish could be predicted with a fair degree of precision.

Under the specified experimental conditions changes in wet weight, the total mass of fatty acids per salmon, and the mass of most individual fatty acids per salmon were dependent on the time in the laboratory and on the product (interaction) of time in laboratory with length of the salmon. At 8-cm length body weight was increasing 30 mg daily and the fatty acid content 7.3 mg daily.

A detailed analysis is presented for several fatty acids whose predicting equations are representative of the different relationships observed. A case is presented for the unique role of fatty acid 22:6 in fish.

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Utilisant des régressions multiples en gradins, nous avons pu formuler des équations exprimant la teneur en acides gras d'alevins de saumon coho, *Oncorhynchus kisutch*, en fonction de la longueur du poisson et du temps pendant lequel il a été maintenu en laboratoire à un régime alimentaire déterminé. De ces régressions, nous avons obtenu de hauts coefficients de corrélation pour la plupart des acides gras étudiés et, à partir des équations ainsi établies, nous avons pu prédire avec assez de précision la teneur en acides gras de poissons individuels.

Dans les conditions expérimentales décrites, les changements de poids humide, la masse totale d'acides gras par saumon et la masse de la plupart des acides gras individuels par saumon sont fonction du temps en laboratoire et du produit (interaction) du temps en laboratoire et de la longueur du saumon. A une longueur de 8 cm, le poids corporel augmente de 30 mg et la teneur en acides gras de 7.3 mg/jour.

Nous présentons une analyse détaillée pour plusieurs acides gras dont les équations sont typiques des diverses fonctions observées. Nous discutons du rôle unique de l'acide gras 22:6 chez les poissons.

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IN our laboratory a study has been made of the manner in which fatty acids are utilized in fingerling salmon exercised at different velocities (Krueger et al. 1968). This was accomplished by comparing the fatty acid content of exercised fish with that of comparable fish which were not exercised. Obviously, a more precise estimate of the utilization of a fatty acid would result if one knew with some certainty the initial amounts of fatty acids in the actual fish which were exercised. This paper summarizes a statistical approach to this problem.

Over the course of the study analyses were made on some 32 salmon from the control group, i.e. those that were not exercised. The information available included their length, weight, the time

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the fish were retained in the laboratory on a specified diet, and total fatty acid content together with fatty acid composition. A stepwise multiple regression was developed to express the quantity of a particular fatty acid in a fish as a function of its length and the time that it was held in the laboratory. Such an analysis has also provided some information on the comparative metabolism of the different fatty acids in the salmon under these experimental conditions.

Experimental

Fingerling salmon, 7.5–8.5 cm, were seined from the Yaquina River near Nashville, Oreg., and transported to the laboratory where they were held in aquaria at 18 C. While in the laboratory (8–31 days) they were fed a diet of small aquatic worms of the genus *Tubifex*. The fatty acid composition of the worms used in this study has been reported previously (Saddler et al. 1966). The salmon were not fed during the 24 hr subsequent to selection as controls or as fish to be exercised.

Lipid from whole fish was extracted using methanol-chloroform and the fatty acids esterified with methanol-HCl. The total amount of methyl esters obtained from each fish was weighed and fatty acid composition was determined by gas chromatography. Details of procedures used in this phase of the analysis have been outlined elsewhere (Saddler et al. 1966).

Stepwise multiple regressions were run for each fatty acid as well as for weight and total mass of fatty acid esters per fish. In such an analysis a regression is run for the dependent variable as a function of the independent variable with which it is most highly correlated. Following this, additional sets of independent variables are entered into the regression equation in a stepwise fashion until an additional variable produced no further reduction in the residual sum of squares. In this fashion a multiple regression is obtained which retains the independent variables which provide a significant input. Computing procedures for this type of analysis have been outlined by Efroymsen (1960).

Results and Discussion

Those quantities to be used as independent variables were specified arbitrarily as length in cm (λ), length squared, time in the laboratory in days (t) as well as this quantity squared, and in addition

TABLE 1. Variables significantly associated with content of individual fatty acids.

Dependent variable	Correlation coefficients (r^2) obtained when independent variables are entered in order from left to right				
	λ^2	$\lambda x t$	t	λ	t^2
weight — g	.760	.862	.876		
Total fatty acids — mg		.788	.875		
Fatty acids:mg					
14:0[a]		.498	.644		
14:1ω6		.544			.640
15:0		.663			.751
15:1ω6		.560	.608		
16:0		.813	.841		
16:1ω7		.798	.823		
16:2ω7		.647			.727
17:1ω9		.698	.724		
18:0		.730	.843		
18:1ω9		.713	.811		
18:2ω6		.668	.794		.818
18:3ω3		.583	.671		
18:4ω3					.486
20:1ω9		.839	.878		
20:2ω6		.579	.667		
20:3ω6		.519	.673		
20:4ω6		.700	.823		
20:5ω3		.724	.805		
22:1ω9					.462
22:5ω3		.699		.813	.856
22:6ω3	.487	.711			

[a]Shorthand designation indicating chain length, number of double bonds, and location of the first double bond counting from the methyl end of the molecule — λ represents the length of the fish and t the time the fish were kept in the laboratory and fed tubificid worms.

an interaction term λxt. Time in the laboratory will obviously influence the amount of fatty acid in the fish, particularly where the fatty acid composition of the diet has an effect. The interaction factor was introduced to account for the fact that the larger fish would consume more food and that food intake would also increase with time.

The extent to which the variation in the dependent variable can be associated with the length of the fish and the time it was held in the laboratory is indicated by the correlation coefficients listed in Table 1. Independent variables which produce a reduction in the residual sum of squares are listed in the order in which they were added to the equation. The progressive increase in r^2 is also noted. Partial regression coefficients which modify these variables were all significantly different from zero ($P < .05$). Several of the regression equations are given in Fig. 2.

The precision with which one can define the fatty acid composition of a fish, knowing its length and time in the lab, is illustrated in Fig. 1, where the calculated values, using the regression equations, are compared with the observed values. A high level of correspondence is observed suggesting that this procedure has some merit in the type of experiments being conducted.

The predicting equations do not describe salmon generally. Rather they describe our salmon between 8 and 31 days when held in tanks and fed tubificid worms. The description is highly dependent on the characteristics of the salmon when caught and the changes developing during the first 8 days in the feeding tank. Completely different relationships would be obtained for example under different dietary conditions.

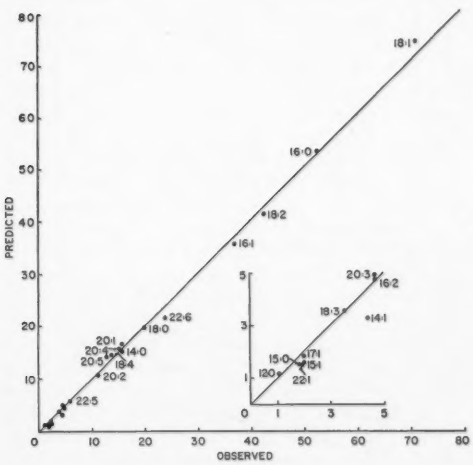

FIG. 1. Comparison of observed values for content of individual fatty acids of a selected salmon with content as obtained from predicting equations involving length of salmon and time salmon was held in laboratory and fed tubificid worms.

To make interpretation of the predicting equation easier, the coordinates in the time–length plane have been altered from (0,0) to (10,7). For t days in the feeding tank there has been substituted $(t+10)$ and for λ cm in length there has been substituted $(\lambda+7)$. Thus, substitution for λ and t in these equations corrects for additional time in the laboratory and for additional final length of the salmon. The predicting equations on Fig. 2 express weights as a function of time beyond 10 days in the laboratory and for fish lengths beyond 7 cm. The ordinates give the weights of specified parameters for salmon and the abscissas, days in the feeding tanks.

The predicting equation for body weight (Fig. 2A) essentially says that weight over the experimental period was a linear function of time if length was kept constant and a quadratic function of length if time was kept constant. The partial derivatives of the regression equation with respect to time and length are

$$\partial W/\partial t = -0.013 + 0.0429\,\lambda$$
$$\partial W/\partial \lambda = 1.659 + 0.176\,\lambda + .0429\,t$$

The daily change in weight depends on the length of the salmon. The change in weight per unit of length increases linearly with both time and length.

The partial derivatives of the predicting equation (Fig. 2B) developed for total fatty acid content per fish are

$$\partial\,(FA)\,/\,\partial t = 2.48 + 4.84\,\lambda$$
$$\partial\,(FA)\,/\,\partial \lambda = 48.4 + 4.84\,t$$

where again t is the time beyond 10 days in the feeding tank and λ is the length beyond 7 cm. The predicting equation for the mass of fatty acids is linear if either length or time is held constant (Fig. 2B; Table 2). The daily change in fatty acid mass depends on the length of the fish and increases about 5 mg for each centimeter in length beyond 7 cm. At 8.0 cm length the gain in fatty acids is one-fourth of the daily gain in wet body weight. The gain in body weight per unit length of fish is related to both time in the laboratory and the length of the salmon, whereas the gain in fatty acid mass per unit length of fish is related only to the time in the laboratory, and is a minimum of 48 mg with approximately an additional 5 mg for each day in the feeding tank.

Of the 21 fatty acids listed in Table 1, 13 have masses increasing linearly with time if length is held constant; and with length if time is held constant. Seven of the fatty acids have a quadratic time factor; five of these seven include the product λxt, and two seem to be independent of salmon length. Only acid 22:6 involved a quadratic length factor.

Some of the data for fatty acids 18:0, 18:2, 18:4, 22:5, and 22:6, derived from the predicting equation and their partial derivatives, are given in Table 2; and prediction equations, modified for a shift of the coordinates in the time–length plane from (0,0) to (10,7), along with curves depicting the changing masses of the fatty acids per fish, are given in Fig. 2C, D, E, and F.

Acid 18:0 was chosen as an example of the acids having a linear prediction equation (Fig. 2C; Table 2) if either length or time were kept constant. The daily rate of gain in 18:0 increased with the

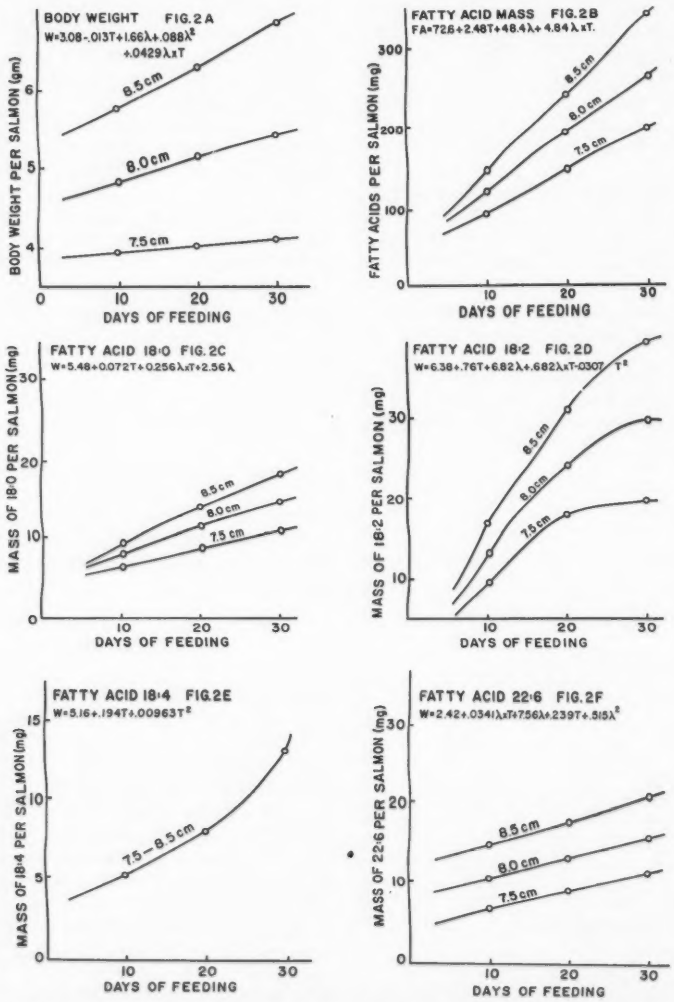

FIG. 2. Body weight and fatty acids per salmon as predicted from equations involving time in laboratory beyond 10 days (t) and length beyond 7 cm (λ).

TABLE 2. Predicted values of body weight and fatty acid content (mg) of 8-cm coho salmon (*Oncorhynchus kisutch*).

			Daily $\partial W/\partial T$	$\partial W/\partial L$	% contribution to total fatty acids	
	Day 10	Day 30	Day 20	Day 20	Day 10	Day 30
Body weight	4.82 g	5.42 g	30 mg	2.26 g	–	–
All fatty acids	121	267	7.3	97	100	100
18:0	8.0	14.6	0.33	5.1	6.6	5.5
18:2	13.2	29.8	0.82	13.6	10.9	11.2
18:4	5.2	12.9	0.39	0.0	4.3	4.8
22:5	2.0	4.5	0.13	2.1	1.7	1.7
22:6	10.5	16.0	0.27	8.9	8.7	6.0

length of the salmon and the increase was about 0.25 mg for each additional cm of length. The minimum increase in 18:0 for each additional centimeter of length beyond 7 cm was 2.56 mg; and to the 2.56 mg was added an additional 0.25 mg for each day the salmon were fed tubificid worms. The proportionate increase in 18:0 was less than that shown by the total fatty acid mass.

Acid 18:2 was one of five fatty acids having a quadratic time component and involving the interaction product λxt. The quadratic time component for all five fatty acids was negative. The predicting equation for 18:2 (Fig. 2D) was linear with respect to fish length if time was held constant and was a quadratic with time if length was held constant. As the quadratic factor was negative the daily rate of increase in the mass of 18:2 per salmon decreased with time, even though the mass of 18:2 accumulated continued to increase with time. The daily rate of increase in 18:2 was further enhanced 0.7 mg for each centimeter, beyond 7 cm, in length, but decreased 0.06 mg/salmon for each day in the tank; the rate of increase was 1.44 mg for an 8-cm fish on day 10 and the rate of increase in 18:2 decreased to 0.21 on day 30. Acid 18:2 increased more rapidly than did 18:0, the ratio of 18:2 to 18:0 on day 10 being 1.64 and on day 30 being 2.04. Acid 18:2 increased at a rate somewhat proportional to that of the total fatty acid mass and its percentage contribution to the total fatty acid mass was not altered much with time.

Acid 22:5 also increased linearly with length if time was held constant, and was a quadratic function of time if length was held constant. For an 8-cm fish the daily increase in the mass of 22:5 was 0.23 mg on day 10 and decreased to less than .02 mg daily on day 30. The rate of change in the mass of 22:5 per unit increase in length was a linear function of time and increased from 1.65 mg per centimeter on day 10 to 2.52 mg on day 30. The percentage contribution of 22:5 to the total fatty acids did not vary markedly with time; acid 22:5 increased more rapidly than 18:0, the ratio of 18:0 to 22:5 on day 10 being 3.96 and 3.22 on day 30. Acid 22:5 increased at a rate similar to that of 18:2, the ratio of 18:2 to 22:5 on day 10 being 6.50 and 6.58 on day 30.

When both t^2 and λxt appeared in the predicting equations, t^2 was negative. When t^2 appeared alone its sign was positive. Only t^2 appeared in the equations for 18:4 and 22:1. The equation and time curve for acid 18:4 are given in Fig. 2E. As the mass of 18:4 does not change with length, only one curve is required. In general a salmon between 7.5 and 8.5 cm after having been fed for 10 days, would have 5.2 mg of 18:4; subsequently the 5.2 mg would be increased around 0.2 mg daily and the 0.2 mg would be subjected daily to an increase around 0.02 mg. The mass of 18:4 per salmon increased proportionately more rapidly than did the masses of 18:0, 18:2, 22:5 or the total fatty acid mass.

Acid 22:6 increased linearly with time if length was held constant and was a quadratic function of length if time was held constant. For a salmon 8 cm in length held 10 days in the feeding tank, the predicted mass of 22:6 was 10.5 mg, or 1.31 mg/cm. The daily gain of 22:6 increased with the length of the salmon. The increase in 22:6 with length of the salmon was greater the greater the length and greater the time the salmon had been fed tubificid worms. For an 8-cm salmon the mass of 22:6/cm of length varied from 1.31 mg on day 10 to 1.99 mg on day 30. Thus the medium-sized salmon of these experiments had increased the mass of 22:6 per unit of length 25% between days 10 and 30.

However, the weight of an 8-cm salmon on day 10 was 4.82 g and on day 30, 5.42 g; the amount of 22:6 changed from 2.75 mg/g to 2.94 mg/g of salmon or only a 7.3% increase in the mass of 22:6/g of salmon. Acid 22:6 increased less rapidly than 18:0, 18:2, 18:4, and 22:5, the ratios of the mass of 22:6 to the other acids being, respectively, 1.25, 0.80, 2.02, and 5.17 on day 10; and 1.10, 0.54, 1.24, and 3.51 on day 30 for an 8-cm salmon.

Previously published data have shown that fatty acid composition varies over a wide range of experimental sequences. We have presented here the pattern of change for a series of fatty acids on the specified diet of tubificid worms. The mass of some of the fatty acids in coho salmon (*Oncorhynchus kistuch*) increased linearly with either time or length of the salmon or both. Some involved quadratic functions of length or of time. While the regression equations have been developed empirically and an exact interpretation of their metabolic significance is not possible at the present, some interesting metabolic effects are suggested.

A very interesting observation from a metabolic point of view is the fact that only in the case of 22:6 is λ^2 a significant contributing factor in the regression equation. The use of λ^2 gave the greatest reduction in variance, and λxt was secondary. One presumes that fatty acid 22:6 is laid down in amounts determined primarily by the size of the fish rather than by the availability of the acid in the diet. In this regard it should be noted that λ^2 is the first variable entering the equation expressing the weight of the fish.

These observations would substantiate other observations which indicate that, compared with other fatty acids, 22:6 is utilized in a unique manner by the fish. This fatty acid is conserved when salmon are allowed to exercise at velocities which the fish can tolerate for extended periods (Krueger et al. 1968). Hanes et al. (1968) have shown that this fatty acid is retained to a much greater extent than other fatty acids in salmon fed restricted amounts of tubificid worms. This selectivity is lost in salmon poisoned with pentachlorophenol (Hanes et al. 1968) or when salmon are exercised at velocities higher than that normally sustained by the fish (Krueger et al. 1968). It is significant that this fatty acid is the major acid of the linolenic acid series which has been shown to be required for normal development of the rainbow trout, *Salmo gairdneri*, (Lee et al. 1967). Subsequent studies have demonstrated that 22:6 is preferentially incorporated in the phospholipids of the rainbow trout (Castell et al. 1972).

The metabolism of 22:6 in the fish has some analogies with the metabolism of arachidonic acid (20:4) in the rat. Compared with other long chain fatty acids arachidonic acid has a considerably lower catabolic rate (Coot 1965). It is postulated that this effect is due to the preferential incorporation of this fatty acid into phospholipids which, because of their incorporation into structural elements in the cell, turn over at rates lower than triglycerides. Possibly 22:6 is playing a comparable role in the fish, the more highly unsaturated character reflecting the lower temperature at which the fish exists.

Another metabolic observation is that this diet produced an increase in the proportion of fat in the salmon and the total quantity of fatty acid methyl esters per fish can be expressed by a regression equation involving λxt, λ, and t. This increase in fat content is doubtless due to the accumulation of triglyceride. One might postulate that those fatty acids whose content is also expressed in terms of λxt, λ, and t represent those fatty acids which are laid down with little selection in the triglyceride under the conditions of this experiment.

Acknowledgments

We wish to acknowledge the assistance of Dr R. G. Petersen of the Department of Statistics.

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Changes in the Population of Lake Whitefish (*Coregonus clupeaformis*) in Lake Winnipeg from 1944 to 1969[1]

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Whitefish catches have fluctuated since 1952, and except in 1964 each successive peak has decreased in magnitude. Recently the overall trend has been a diminishing fishery with increasing effort.

There has been a drastic change in age composition with time. Age-groups 5, 6, and 7 accounted for 81% of the catch from 1944 to 1948, age-groups 4, 5, and 6 for 89% from 1949 to 1955, age-groups 4 and 5 for 86% from 1959 to 1968, and age-groups 3 and 4 for 88% in 1969. The fishery is now dependent upon two age-groups instead of three. Natural failure of one or more important year-classes would result in a serious decline in catch, and partial or complete collapse of this fishery.

Annual survival rate decreased from 34%, 1944–48 to 12%, 1959–69. Instantaneous natural mortality rate was estimated to be 0.72, and may be overestimated. The 1938–39, 1945–47, 1949, and 1957 year-classes were strong, while the 1940–44, and 1956, 1958, and 1961 year-classes were weak. The 1959 and 1960 year-classes appeared to be somewhat above average. Examination of data on total dissolved solids and temperature indicated that ecological changes were not responsible for the decline of whitefish in Lake Winnipeg.

We judge that the optimum rate of fishing has been exceeded, and that the fishery itself is largely responsible for the depleted condition of the whitefish stocks in Lake Winnipeg. Fishing effort must be reduced to the point where the fishery is again dependent upon three year-classes. This should result in higher catches, and provide less fluctuation in annual catches.

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Les prises de grand corégone ont subi des fluctuations depuis 1952, et sauf en 1964 chaque sommet successif a été moins élevé que le précédent. Récemment, la tendance générale a été vers une diminution des prises et une augmentation de l'effort.

Il y a eu avec le temps un changement dramatique de la proportion des âges dans la population. Les classes d'âge 5, 6 et 7 ont été responsables de 81% des prises entre 1944 et 1948, les classes d'âge 4, 5 et 6 de 89% entre 1949 et 1955, les classes d'âge 4 et 5 de 86% entre 1959 et 1968, et les classes d,âge 3 et 4 de 88% en 1969. La pêcherie repose maintenant sur deux classes d'âge au lieu de trois. L'insuccès naturel d'une ou de plusieurs classes d'âge pourrait donc causer une diminution sérieuse des prises et un affaissement partiel ou total de cette pêcherie.

Le taux annuel de survie a passé de 34% en 1944–1948 à 12% en 1959–1969. Le taux instantané de mortalité naturelle a été estimé à 0.72, mais peut avoir été surestimé. Les classes d'âge de 1938–1939, 1945–1947, 1949 et 1957 étaient abondantes, alors que celles de 1940–1944 et celles de 1956, 1958 et 1961 étaient faibles. Les classes d'âge de 1959 et 1960 ont semblé quelque peu au-dessus de la moyenne. L'analyse des données relatives aux solides totaux dissous et à la température démontre qu'on ne peut attribuer aux changements écologiques le déclin du grand corégone dans le lac Winnipeg.

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Nous croyons que le taux optimal d'exploitation a été dépassé, et que la pêche elle-même est en grande partie responsable de l'épuisement des stocks de grands corégones du lac Winnipeg. On devra réduire l'effort de pêche à un niveau où la pêcherie sera de nouveau alimentée par trois classes d'âge. Il devrait en résulter des prises plus fortes et des fluctuations moins grandes des prises annuelles.

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THE lake whitefish, *Coregonus clupeaformis*, is one of several commercial fish species present in Lake Winnipeg. Whitefish are used to supply the smoked fish trade and gefillte fish industry in the United States. The main commercial fishery for lake whitefish in Lake Winnipeg is conducted in the north end of Lake Winnipeg during the summer season. Fishing normally begins early in June, as soon as the lake is free of ice, and ends normally about the first week in August or earlier, depending upon the specific regulations set each year.

The commercial fishery uses gillnets as the principal gear. The nets used in the summer fishery have been mainly 5- or 5¼-inch stretched measure. A detailed description of this fishery has been published by Kennedy (1954).

The first records of commercial whitefish production from Lake Winnipeg date back to 1883. For over 50 years the lake has produced an average of about 3,000,000 pounds of whitefish annually (Hewson 1959). The annual catch ranged from a high of 7.5 million lb in 1904 to a low of 0.7 million lb in 1936 (Hewson 1960). The largest catches in the past 10 years have barely exceeded the smallest catches in former years, except for 1936. The trend of declining whitefish catches has caused considerable concern in industry and government circles. The availability of size and age composition data collected over the period 1944–69 provided the opportunity of examining the population dynamics of whitefish. The purpose of this paper is to measure and document the effect of the fishery on this population, to examine the potential causes responsible for the drastic decline in whitefish production in Lake Winnipeg, and to suggest measures for sound management of this important fishery. The whitefish population has shown definite trends in catch, effort, catch per unit of effort, age composition, and mortality over the past 26 years. These subjects plus year-class strength are examined in detail.

Sampling Methods

Age data on Lake Winnipeg whitefish were available from the period 1937 to the present time, with the exception of the period 1956–58 inclusive. However, data prior to 1944 were not considered representative by Kennedy (1954). Therefore, data prior to 1944 were excluded from this present analysis.

Kennedy (1954) stated that length, weight, and scale samples obtained during the period 1944–51 were collected from all fish used in the annual routine inspection for possible infection with *Triaenophorus*. Fish chosen for sampling were taken from places widely distributed over the entire fishing grounds (Fig. 1 and Table 1). Fishing was conducted mainly around the areas where fishing stations exist. Instructions for the collection of these samples were designed to ensure random samples being obtained at each collection site. Samples were taken generally once a year, at each fishing station during the summer fishing season. Samples collected during the period 1944–51 inclusive were obtained from fish landed at 14 different fishing ports. However, they were taken consistently at the following six locations: Berens River, George Island, Sandy Island, Big Black River, Spider Island, and Warren Landing (Table 1). This table lists the total number collected each year from each station for age determination.

In 1948, the Central Station of the Fisheries Research Board of Canada initiated an investigation of the commercial fish of Lake Winnipeg, especially the lake whitefish. One of the objectives of this study was to establish trends in the population of whitefish, to predict the catch in future years.

FIG. 1. North basin of Lake Winnipeg showing localities mentioned in the text.

TABLE 1. Numbers of lake whitefish (*Coregonus clupeaformis*) sampled for age determination from commercial catches of Lake Winnipeg summer whitefish fishery by year and area.

	Area Sampled															
Year	Berens River	Reindeer Island	George Island	Sandy Island	Poplar Point	Big Black River	Shoal Point	Spider Island	Warren Landing	Eagle Island	Birch Island	Grand Rapids	Horse Island	Little George Island	Montreal Point	Total
1944	98	100	100	100	84	99	99	100	102	100	–	–	–	–	–	982
1945	98	–	95	100	98	98	–	95	96	–	99	–	–	–	–	779
1946	97	–	97	90	–	99	97	96	–	–	–	99	–	–	–	675
1947	98	–	98	100	90	99	100	100	98	–	–	–	32	–	–	815
1948	149	–	150	150	–	137	149	149	149	–	–	–	147	–	–	1180
1949	98	–	99	70	–	100	–	100	100	–	–	–	96	–	–	663
1950	97	–	87	98	–	102	–	98	200	–	–	–	–	97	–	779
1951	100	–	99	100	–	99	–	97	98	–	–	–	–	–	–	593
1952	506	–	–	–	–	530	–	–	517	–	–	–	–	–	–	1553
1953	572	–	–	–	–	511	–	–	520	–	–	–	–	–	–	1603
1954[a]	302	–	–	–	–	267	–	141	263	–	–	–	–	–	–	973
1955[a]	302	–	–	–	–	353	–	–	170	–	–	–	–	–	–	825
1956	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1957	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1958	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1959	–	–	652	149	492	461	–	–	1094	–	–	–	827	–	–	3675
1960	–	–	499	–	–	917	–	–	972	–	–	–	1049	–	246	3683
1961	–	–	–	–	–	505	–	–	462	–	–	–	530	–	–	1497
1962	–	–	515	–	–	918	–	–	866	–	–	–	509	–	–	2808
1963	–	–	1011	–	–	1164	–	–	999	–	–	–	936	–	–	4110
1964	–	–	1020	–	–	960	–	–	915	–	–	–	1006	–	–	3901
1965	–	–	960	–	–	996	–	–	994	–	–	–	1007	–	–	3957
1966	–	–	497	–	–	–	–	–	503	–	–	–	503	–	–	1503
1967	–	–	1003	–	–	–	–	–	1000	–	–	–	1000	–	–	3003
1968	–	–	501	–	505	–	–	–	1001	–	–	–	1011	–	–	3018
1969	–	–	–	–	987	–	–	1004	1006	–	–	–	–	–	–	2997

[a]Exclusive of 4½-inch and 4¾-inch mesh nets.

Representative samples of the commercial summer whitefish catch were collected during half-month periods, to gain information on the size composition of the commercial catch. Fish were selected at random from the catches while the boats were unloaded, and each fish was weighed individually on a small spring scale (Hewson 1959). During the years 1952 and 1953, whitefish scale samples for age determination studies were obtained by randomly sampling the commercial catches of whitefish landed at Warren Landing, Big Black and Berens rivers (Table 1). Sampling at these stations was continued in 1954 and 1955, by employees of the Manitoba Government. In addition, fish were also sampled at Spider Island in 1954. Sampling was discontinued during the years 1956–58 inclusive.

With the recommencement of sampling in 1959 by employees of the Manitoba Government, data were collected in more detail. Sampling of the commercial catch was intensified, and a concerted effort was made to sample the same fishing stations each year. Scale samples were collected generally twice during the season, in June and July, at three or more fishing stations. In recent years, these have been Warren Landing, Big Black River, George Island, and Selkirk or Horse Island.

In addition, manuscript reports Doan (1959), Rybicki (1961, 1962, 1963, 1964, 1965), Rybicki and Doan (1966), Howard (1972), and Pollard (1972) were written each year, which described the age composition of the commercial catch including data on the average weight and size of fish at each age. In addition, various aspects of the age, growth, catch, effort, and catch per unit of effort of whitefish were discussed in detail. Thus, the period from 1959 to 1969 contained the most abundant data for analysis of the whitefish summer fishery on Lake Winnipeg.

The numbers of whitefish sampled each year for age determination varied considerably. Annual totals ranged from a low of 593 in 1951, to a high of 4110 in 1963 (Table 1). Age composition data are shown in Table 2. These data form the basic data for this present analysis.

Examination of the basic age composition data used in Rybicki and Doan (1966) indicated that age composition of whitefish varied among different stations sampled within the same month and year, as well as between months in the same year. Age composition data for each year in the series of years 1959–63 were examined in detail. For each year, monthly age composition data at each station were weighted by the corresponding monthly catch at each station, before combining to obtain monthly and annual estimates of age composition for the entire fishery. Comparison of weighted data with unweighted data showed no apparent difference. Tagging studies (Pollard 1973) showed that whitefish tagged at Warren Landing were recaptured throughout the most important summer whitefish grounds. These results indicate that the same population of whitefish is exploited throughout the north basin of Lake Winnipeg. In view of these findings, scale samples collected from all stations during the same year were combined to obtain a single sample for age composition studies.

Average weight data for individual years in the period 1944–51 were obtained by weighting the average weight for each age in a given year (Kennedy 1954) by the corresponding number of fish of that age. However, Kennedy did not compute average weights for samples containing less than 20 fish; therefore, the computed annual mean weights probably were slightly underestimated, since most of the fish excluded by Kennedy were in the older age groups. Average weights of whitefish in the years 1952–54 were calculated from data in Hewson (1959) for areas B and D. Data for subsequent years were obtained from the following: Kooyman (1955), Doan (1959), Rybicki (1961, 1962, 1963, 1964, 1965), Howard (1972), and Pollard (1972). These data were utilized, together with catch data (round weight), to estimate the total number of fish caught each year (Table 3).

Catch data for the summer whitefish fishery for the period 1944–69 are shown in Table 3. These data were recorded originally as dressed weight, which was the form in which they were delivered and sold to the fishing companies. These data were converted to round weight, assuming a weight loss of 12% from round to dressed (Doan 1959).

Employing procedures used by Rybicki and Doan (1966), a rough measure of fishing effort in standard days was obtained. The number of licenses issued each season was multiplied by the number of days in the fishing season, and by efficiency factors for changes in mesh size, amount of nets (yardage) fished, and net type (Table 4). Efficiency factors of 1.0 for 5¼-inch mesh nylon nets and 1.5 for 5-inch mesh nylon nets were employed, based on the results of Kooyman (1955), who found no significant difference in the size, weight, age, or maturity of fish taken in 5-inch, 5⅛-inch, or 5¼-inch mesh nets. Smaller mesh nets, 4¼ inches and 4⅜ inches were found to be even more efficient, which is common knowledge among experienced fishermen. The number of net yards legally permitted each fisherman was weighted by efficiency factors of 1.0 and 1.6 for 5000 and 8000 yards of net, respectively. Efficiency factors of 3.0 for nylon nets and 1.0 for cotton nets were employed, based on the work of Lawler (1950), McCombie and Fry (1960). In addition, all effort data were multiplied by a constant scaling factor of 0.13, to make the catch per effort a more meaningful measure of the average daily catch of whitefish by individual fishermen.

This constant was arrived at in the following manner. Annual records of weekly whitefish catches landed by each fishing boat were obtained from weekly reports of whitefish landings sent in by fish station operators to the Manitoba government. Employing these data, the mean annual whitefish catch in pounds per boat day was calculated for each year in the series of years 1959–63. A mean annual whitefish catch figure of 201.4 lb/boat day was calculated for this 5-year period from the annual means. This figure was then compared with the mean annual whitefish catch of 26.4 lb/standard day, which was estimated for the same period. This figure was obtained from the data in Table 4, employing the standardization of effort methods described above, but excluding the constant scaling factor of 0.13. Comparison of these two means indicated that the mean catch per boat day estimate was 7.6 times greater than the mean catch per standard day estimate. The data used to

TABLE 2. Annual age composition of lake whitefish sampled from commercial catches in Lake Winnipeg summer whitefish fishery, 1944–55 and 1959–69.

	Year of capture																						
Age-group	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969
1																1							
2													3	11	7	4					1	1	74
3			1			2	1	1	7	18	3		100	287	106	110	298	76	161	180	115	206	1456
4	19	3	18	19	33	225	114	78	108	651	25	34	1590	1236	851	850	1797	1389	1274	602	1485	916	1182
5	330	42	79	157	335	172	409	277	1141	298	421	285	1612	1784	431	1642	1402	2111	2228	589	1309	1662	240
6	401	267	143	287	507	130	151	192	241	548	396	403	329	293	79	179	513	298	278	123	85	203	34
7	156	322	235	202	190	77	72	32	49	68	95	96	33	44	11	18	73	19	11	8	8	27	9
8	45	114	145	96	59	26	19	9	5	13	27	4	4	21	9	3	18	6	3	1		3	1
9	16	21	39	36	41	14	5	2	2	2	4	1	3	4	3	1	5	1	2				
10	4	8	9	10	9	10	3			2	2	1		2			4						1
11	6	1	5	4	4	5	3	2					1	1				1					
12	3		1	2	1	2	1			2		1											
13		1					1																
14				1						1													
15	1			1																			
16	1																						
17					1																		
Total	982	779	675	815	1180	663	779	593	1553	1603	973	825	3675	3683	1497	2808	4110	3901	3957	1503	3003	3018	2997

calculate standard fishing effort in Table 4 would have to be multiplied by 0.13, the reciprocal of 7.6, to obtain identical means. Therefore, all effort data in Table 4 was multiplied by a factor of 0.13.

Survival rates were estimated by the Robson and Chapman (1961) method. In some cases, their method of analysis when age was known for the entire sample was employed, while in other cases, either their method of analysis when some age-groups were combined or methods of analyzing a segment of a catch curve were used. In each method, year-class strength and annual survival rate were assumed to be constant, at least over a limited range of age-groups. In addition, all age-groups beyond some minimum age were assumed to be equally vulnerable to the sampling gear.

Estimates of year-class strength were made by the virtual population method of Fry (1949). He defined the virtual population as the sum of the fish belonging to a given year-class present in the water at any given time that are destined to be captured in the fishery in that year and all subsequent years.

Catch and Effort

FIG. 2 compares total catch, effort, and catch-per-effort for the summer whitefish fishery, for the period 1944–69; this period had the most detailed data. Examination of these catch data indicates that the greatest catch was in 1945, when over 2.2 million lb of whitefish were landed. A poor catch occurred 3 years later in 1948, when approximately 1.1 million lb of whitefish were caught. The next peak catch occurred in 1950, when a little less than 2.2 million lb were caught, while in 1952 slightly more than 2.0 million lb were caught. Catches have fluctuated since 1952, but each successive peak has decreased in magnitude, with the exception of that in 1964. The lowest catches occurred in 1968 and 1969, when 0.7 and 0.5 million lb of whitefish were caught, respectively. Fishing intensity generally increased from 1944–68, indicating that the decline in catch over this period is not due to lack of effort. Catch-per-day-data further substantiate the decline in abundance. The large whitefish catches from 1950–52 can be attributed partly to the increased efficiency of the fishery, caused by the change from cotton to nylon gillnets, as well as the presence of strong year-classes.

Effort was calculated as described previously, using data on number of licenses issued each season, season length, and efficiency factors for changes in mesh size, number of net yards fished, and net type.

TABLE 3. Annual total catch, average weight, and estimated numbers of lake whitefish in Lake Winnipeg summer whitefish fishery, 1944–55 and 1959–69.

Year	Summer production (lb dressed)	Summer production (lb round)[a]	Average weight (lb round)	Estimated numbers of fish
1944	1,885,814	2,143,039	2.5	857,216
1945	2,239,085	2,544,496	2.6	978,652
1946	2,114,822	2,403,284	2.7	890,105
1947	1,245,828	1,415,759	2.7	524,355
1948	1,106,560	1,257,495	2.8	449,105
1949	1,353,427	1,538,034	2.3	668,710
1950	2,171,274	2,467,436	2.4	1,028,098
1951	1,949,352	2,215,244	2.4	923,018
1952	2,026,447	2,302,854	2.5	921,142
1953	1,228,774	1,396,379	2.4	581,824
1954	1,297,245	1,474,189	2.4	614,245
1955	1,583,517	1,799,509	2.4	749,795
1959	1,288,579	1,464,341	2.2	665,610
1960	869,595	988,208	2.3	429,656
1961	993,993	1,129,574	2.2	513,443
1962	1,180,618	1,341,654	2.3	583,328
1963	982,400	1,116,399	2.3	485,391
1964	1,291,222	1,467,345	2.3	637,976
1965	1,090,744	1,239,521	2.2	563,419
1966	916,867	1,041,928	2.1	496,156
1967	1,144,100	1,300,155	2.1	619,121
1968	723,000	821,617	2.3	357,225
1969	502,623	571,181	1.7	335,989

[a]Conversion factor dressed to round, 1.1364.

TABLE 4. Total catch, effort in standard days, and catch per effort of lake whitefish in Lake Winnipeg summer whitefish fishery, 1944–69.

Year	Summer production (lb dressed)	Number of licences	× Length of season (days)	× Mesh size efficiency factor	× Yardage efficiency factor	× Net type efficiency factor	= Standard days	Catch per standard day (lb dressed)
1944	1,855,814	150	53	1.0	1.0×0.13	1.0	1034	1825
1945	2,239,085	150	53	1.0	1.0×0.13	1.0	1034	2167
1946	2,114,822	150	53	1.0	1.0×0.13	1.0	1034	2046
1947	1,245,828	150	53	1.0	1.0×0.13	1.0	1034	1205
1948	1,106,560	150	53	1.0	1.0×0.13	1.0	1034	1071
1949	1,353,427	133	53	1.0	1.0×0.13	1.5	1374	985
1950	2,171,274	150	53	1.0	1.0×0.13	2.5	2584	840
1951	1,949,352	150	53	1.0	1.0×0.13	3.0	3100	629
1952	2,026,447	149	44	1.0	1.0×0.13	3.0	2557	793
1953	1,228,774	132	62	1.0	1.0×0.13	3.0	3192	385
1954	1,297,245	131	68	1.0	1.0×0.13	3.0	3474	373
1955	1,583,517	130	70	1.0	1.0×0.13	3.0	3549	446
1956	1,031,741	126	70	1.5	1.0×0.13	3.0	5160	200
1957	1,346,031	98	70	1.5	1.0×0.13	3.0	4013	335
1958	823,000	85	76	1.5	1.0×0.13	3.0	3779	218
1959	1,288,579	88	69	1.5	1.6×0.13	3.0	5683	227
1960	869,595	83	69	1.5	1.6×0.13	3.0	5360	162
1961	993,993	76	69	1.5	1.6×0.13	3.0	4908	203
1962	1,180,618	80	69	1.5	1.6×0.13	3.0	5167	228
1963	982,400	78	69	1.5	1.6×0.13	3.0	5038	195
1964	1,291,222	80	69	1.5	1.6×0.13	3.0	5167	249
1965	1,090,744	78	69	1.5	1.6×0.13	3.0	5038	217
1966	916,867	79	69	1.5	1.6×0.13	3.0	5102	180
1967	1,144,100	76	80	1.5	1.6×0.13	3.0	5691	201
1968	723,000	75	69	1.5	1.6×0.13	3.0	4844	149
1969	502,623	44	69	1.5	1.6×0.13	3.0	2842	177

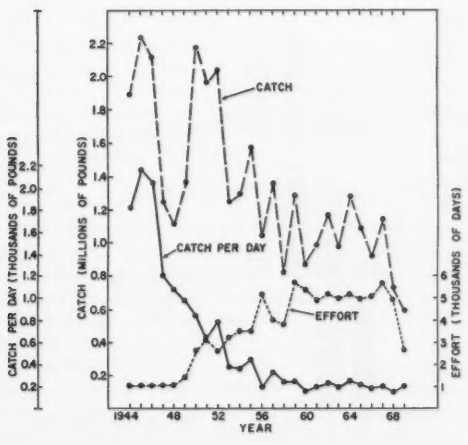


FIG. 2. Total catch, effort, and catch per effort, of whitefish in Lake Winnipeg summer whitefish fishery, 1944–69.

The quantities of effort calculated by this method are at best estimates, since the number of people fishing, the number of days fished by each fisherman, and the quantity and mesh size of gillnets fished by each fisherman are unknown. Restrictions imposed by fishing companies limiting or restricting deliveries would further limit catch and effort, and affect this method of estimating effort. However, the method just outlined is the best presently available.

It should be noted that the changes that occurred in the fishery from 1944–69 divide the fishery into four different periods according to time — 1944–48, 1949–55, 1956–58, and 1959–69. The first period, 1944–48, comprises a 5-year period when fishing regulations were constant: one hundred and fifty licenses each season, 53 day season, and 5000 yards of cotton gillnet 5¼-inch stretched measure the legal gear for each fisherman.

The second period, 1949–55, resulted from a change in fishing gear to nylon nets, which had three times the efficiency of cotton nets (Lawler 1950). The third period, 1956–58, resulted from a further

change in the fishing regulations, whereby the legal mesh size was reduced in 1956 from 5¼-inch to 5-inch stretched measure. The 5-inch mesh was estimated to be 1.5 times as efficient as the 5¼-inch mesh (Kooyman 1955). The last change in the fishing regulations occurred in 1959, when the legal quantity of gillnets fished by an individual fisherman was increased from 5000 to 8000 yards. By 1959, most of the fishing boats were equipped with power net lifters.

The graph of fishing intensity (Fig. 2) shows a small amount of effort from 1944–48. The increased effort from 1949–55 was partly the result of the increased efficiency of nylon nets over cotton nets. The peak in 1956 coincided with the change to a smaller mesh size in 1956. The decrease in 1958 reflects the decrease in the number of licenses from 126 in 1956 to 85 in 1958. The increase in 1959 reflects the increase from 5000 to 8000 yards per license, which took effect in 1959. The decline in 1969 resulted from the decrease in the number of licenses issued (Table 4).

Comparing the catch and effort curves, the catch data show a trend of declining catch after 1952, while the effort data show a trend of increasing effort with time from 1949 to 1968. The catch-per-day data further illustrate the decline in abundance following 1952. Thus, the decline in catch is not the result of lower fishing effort, but of overfishing.

Age Composition

Age composition data for each year in the series of years, 1944–69 (Table 2), were examined in detail. The accuracy of age determination decreases with the increasing age of the fish. For purposes of analysis, it was generally assumed that fish collected during the years 1944–50 could be aged accurately through age 11 or 12, while fish collected during the years 1951–55 and 1959–69 could be aged accurately through age 9 or 10. In determining the accuracy of the above age determinations, consideration was given to the relative strength of each year-class. It was assumed that strong year-classes could be traced for a longer period than weak ones.

The average percentage age composition for each fishing period and for the year 1969 are shown in Fig. 3. The age composition data show a drastic change in age composition with time. Intensified fishing effort and reduction in the mesh size of nets fished has brought about the exploitation of younger and younger age-groups of whitefish. Age-groups 5, 6, and 7 accounted for 81% of the catch from 1944 to 1948, age-groups 4, 5, and 6 for 89% from 1949 to 1955, age-groups 4 and 5 for 86% from 1959 to 1968, and age-groups 3 and 4 for 88% in 1969.

Changes in age composition were accompanied by decreases in average weight during these periods. The average weight of whitefish was 2.7 lb from 1944 to 1948, 2.4 lb from 1949 to 1955, 2.2 lb from 1959 to 1968, and 1.7 lb in 1969 (Table 3).

Hewson (1959) in his 7-year study of the summer whitefish fishery, 1948–55, remarked that the 5¼-inch legal mesh gillnets were no longer used exclusively for whitefish. Illegal gillnets as small as 4½-inch stretched measure gradually had become common. Thus, it is no surprise that fish as young as 3 years of age appeared in the commercial catches. In 1969, 3-year-olds accounted for 49% of the commercial catch. Miller (1956) concluded from his study of Pigeon Lake, Alta. that heavy fishing contributes to the likelihood of collapse of a fishery by reducing the number of year-classes that make up each year's catch. When exploitation is light enough so that four or more year-classes contribute to each year's harvest, then the failure of a single year-class has little effect on the fishery.

The Lake Winnipeg whitefish fishery is now dependent upon two age-groups instead of three. Natural failure of one or more important year-

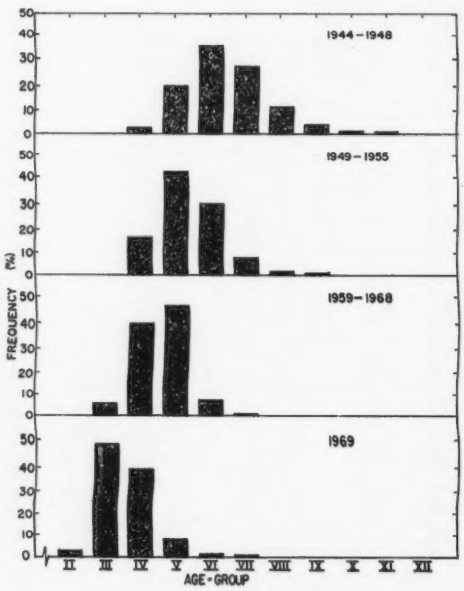


FIG. 3. Average percentage age composition of whitefish in Lake Winnipeg commercial catch during different periods.

classes would result in a serious decline in catch, and partial or complete collapse of this fishery.

During the period 1959–69, the fishery became dependent upon two instead of three year-classes. This event, coupled with the change in age composition which resulted in dependence on the 4- and 5-year-olds, may affect the reproductive potential of the stock, and result in further decline. Howard (1972) found that over 40% of the spawning stock consisted of 4-year-old fish, which were spawning for the first time. Thus, the commercial fishery is placing considerable fishing pressure on prespawners and spawners. Christie and Regier (1972), following Abrosov's approach in their analysis of lake whitefish data from Lake Ontario suggested that a maturing lake whitefish should on the average be assured the opportunity of spawning at least 1.5 times, to enable the population to maintain itself. Many Lake Winnipeg whitefish have not been allowed the opportunity to spawn at least once. This was especially true in 1969, when approximately 49% of the total number of fish caught were 3-year-olds, and 39% were 4-year-olds. The low abundance of whitefish 5 years of age and older (9% of the total catch) may have placed the whitefish stock in grave danger, since the number of mature fish available for spawning in 1969 was considerably reduced. The abundance of 4-year-old fish in 1973 should provide further insight as to whether the spawning stock was too small in 1969.

Fishing effort must be reduced to the point where the fishery is again dependent upon three year-classes instead of two. This should result in higher catches, and provide less fluctuation in annual catches. More whitefish should be assured the opportunity of spawning at least once. During years when weak year-classes are predominant in the fishery, special protection from fishing must be afforded these stocks, to permit adequate escapement for spawning.

Annual Survival Rate

Age composition data for each year in the series of years 1944–69 were examined in detail. The catch curves for each year are shown in Fig. 4. The catch curves show a change in age composition with time, with complete disappearance of fish older than age eight by 1966, except for 1969.

Note that the catch curve in 1949 has a sinuous shape, while catch curves in other years are concave, especially during the period 1959–66. The concave curves probably represent a temporary phenomenon resulting from increased fishing effort beginning in 1949. The concave curves suggest that the older ages, age eight and older, have not passed through their recruitment period at the new mortality rates, and still reflect the previous lower mortality rate. This would particularly apply to the 1959–63 curves, assuming fish entered the catch as 4-year-olds. The curves from 1964 to 1966 might well represent a period of transition, while the curves in 1967 and 1968 suggest the population has stabilized at the new rate.

One major problem involved in analyzing these data has been determining the age at which the whitefish become fully vulnerable to the fishing gear. This has been further complicated by the use of illegal small mesh nets, which increase the vulnerability of younger ages. In estimating the annual survival rate, the youngest vulnerable age was determined initially by inspection of the catch curve. In some cases, the entire right descending limb of the catch curve was used, and in others, segments of the catch curve were used for estimating survival rates, because of the concave shape of the catch curves in some years and their sinuous shape in other years (Table 5). Survival rates were estimated by the method of Robson and Chapman (1961). Where segments were used, the steepest portion of the catch curve was used to obtain the most recent survival rate. Chi-square goodness-of-fit tests (D. S. Robson personal communication) were utilized to test whether survival rates between different ages in the same segment were similar. This test establishes whether or not reasonable agreement exists between the observed frequency in age-group 0 and the frequency expected on the basis of data in the older age-groups. A significant value of chi-square indicates that one of the assumptions of the basic model has not been met. The test does not identify which basic assumption has not been met. In every case where a significant value of chi-square was obtained, the age-group coded as 0 was eliminated from the data. The remaining data were then recoded, and a new estimate computed and tested by means of a chi-square test. The same procedure was repeated, if the next chi-square test was found to be significant. The resultant estimates are subject to error, since the basic assumptions may not have been met in all cases. However, the general decrease in survival rate is believed to be real, and is borne out by the increased amount of fishing effort in recent years, as well as other factors, such as exploitation of younger age-groups. The basic age composition data are listed in Table 2, while the age-groups employed and estimated annual survival rates are listed in Table 5.

Examination of the estimated annual survival rates in Table 5 indicates that considerable fluctuation has occurred in the annual survival rate. During the first period, 1944–48, the survival rates were

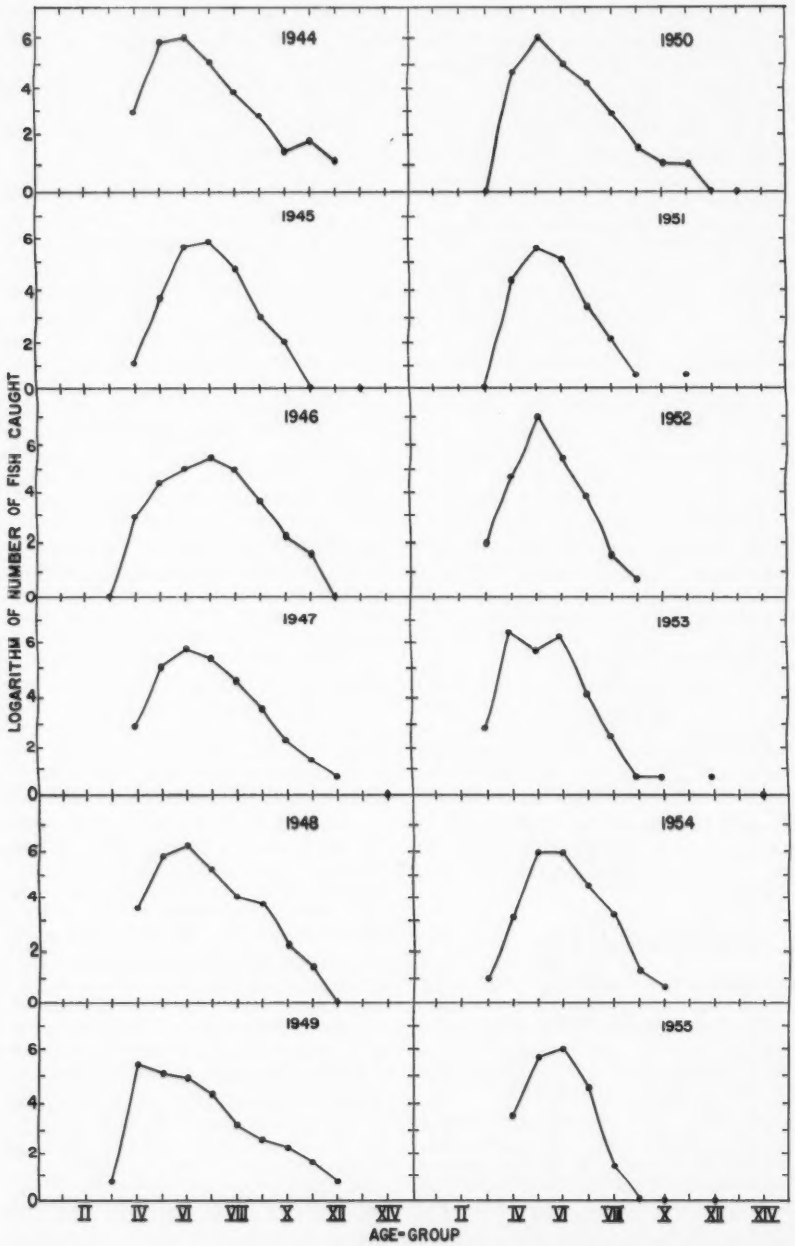
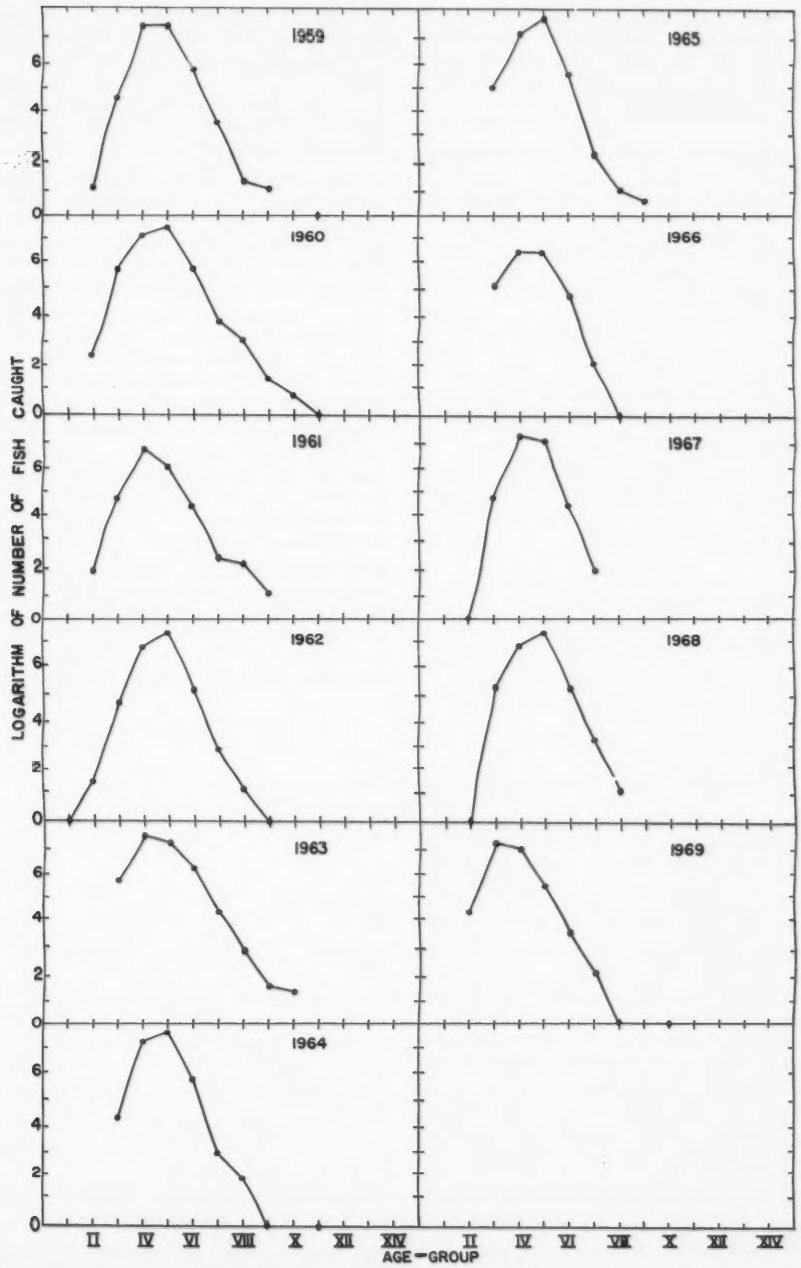


FIG. 4. Annual catch curve for whitefish in Lake Winnipeg, summer white-



fish fishery, 1944–55, 1959–69. Whitefish older than age XIV are omitted.

TABLE 5. Annual survival rates estimated by Robson-Chapman method and corresponding instantaneous total mortality rates for whitefish in Lake Winnipeg summer whitefish fishery. Two standard errors are in parentheses. G. indicates data are grouped.

Year	Segment of catch curve[a]	Survival rate (s)	Instantaneous total mortality (i)
1944	6–10	0.343(±0.033)	1.07
1945	7–11	0.288(±0.037)	1.24
1946	8–12G	0.277(±0.054)	1.28
1947	7–12	0.410(±0.047)	0.89
1948	6–12	0.381(±0.028)	0.96
1949	6–12	0.498(±0.048)	0.70
1950	5–10	0.377(±0.031)	0.98
1951	6–9	0.196(±0.048)	1.63
1952	5–9	0.201(±0.019)	1.60
1953	6–9	0.138(±0.026)	1.98
1954	6–9	0.244(±0.035)	1.41
1955	7–9	0.057(±0.045)	2.87
1959	6–8	0.103(±0.031)	2.27
1960	5–7	0.161(±0.016)	1.83
1961	5–7	0.173(±0.033)	1.75
1962	5–9	0.110(±0.014)	2.21
1963	6–9	0.172(±0.028)	1.76
1964	6–7[b]	0.064(±0.030)	2.75
1965	6–7[b]	0.040(±0.024)	3.24
1966	6–8	0.071(±0.043)	2.65
1967	5–7	0.067(±0.013)	2.70
1968	5–8	0.123(±0.014)	2.09
1969	4–8	0.189(±0.018)	1.67

[a]Catch curves are shown in Fig. 4.
[b]Other portions of curve significantly different.

generally high, and ranged from 28–41%, average 34%. This is comparable to Kennedy's (1954) estimate of 36% for the 1944–51 grouped data.

During the second period, 1949–55, the estimated annual survival rates decreased somewhat, except for 1949. Annual survival rates ranged from 6 to 50%, average 24%. The generally lower survival rates in the second period compared to the first period reflect the increased efficiency of the fishery, resulting from the change from cotton to nylon mesh nets, as well as the increase in season length, beginning in 1953. The high rates in 1949 and 1950 may be biased by differences in year-class strength.

During the last period, 1959–69, the estimated annual survival rates were generally lower than those in the second period. Annual survival rates during the third period ranged from 4 to 19%, average 12%. The high survival rate in 1969 reflects the drop in total fishing effort caused by the reduction in the number of licensed fishermen. In addition, the increased use of small mesh nets by fishermen probably decreased the fishing pressure on the older age-groups, since the 3-year-old age-group was fished quite intensively in 1969. In summary, there has been approximately a threefold decrease in average annual survival rate from 34 to 12%. This sharp decline in survival rate reflects the result of intense overfishing.

Each annual estimate of total mortality was plotted against it's corresponding annual value of fishing effort (in Fig. 5), to determine whether the total mortality rate was correlated with fishing effort, and to obtain an estimate of the natural mortality rate. The data indicate a positive correlation. A least-squares regression line was fitted to the points, to obtain an estimate of the natural mortality rate. The estimated regression equation was $Y = 0.7165 + 0.0032X$, where Y is the annual instantaneous total mortality rate and X is the annual fishing effort in thousands of standard days. The estimated instantaneous natural mortality rate of 0.72 corresponds to an annual natural mortality rate of 51%. This rate is considerably more than that reported by Neth (1959), who found a natural mortality rate of 22% in an unexploited population in a small Adirondack Lake in New York. Our rate is similar to those reported by Kennedy (1953, 1954, 1963), who reported total annual mortality rates of 57–81% in four lightly fished lakes in northern Canada, and 64% in Lake Winnipeg for the period 1944–51. Rybicki and Doan (1966) esti-

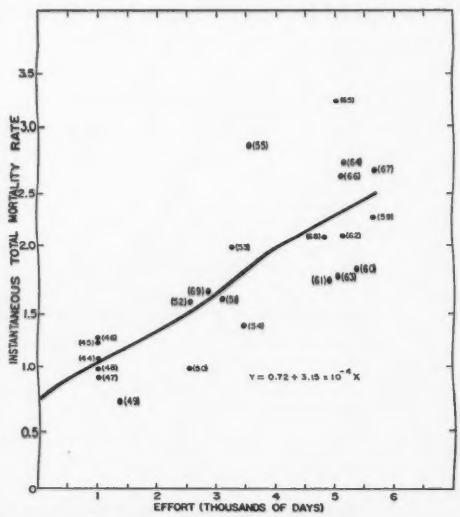


FIG. 5. Comparison of annual fishing effort and instantaneous total mortality rate for the years 1944–55 and 1959–69.

TABLE 6. Numbers (in thousands) of lake whitefish caught in Lake Winnipeg summer whitefish fishery by year and year-class.

	Year of Catch																									
Year-class	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	Virtual population
1932[a]	3																										3
1933[a]	5																										5
1934[a]	4	1																									5
1935[a]	14	10	7	1																							32
1936[a]	39	26	12	3																							80
1937[a]	136	143	51	7	2	2																					341
1938[a]	350	405	191	23	3	5																					977
1939[a]	288	335	310	62	16	10	4																				1025
1940	17	53	188	130	22	14	4																				428
1941		4	104	185	72	26	7																				398
1942			24	101	193	78	25	3																			424
1943			1	12	128	131	95	14	1																		382
1944					13	173	199	50	3	1																	439
1945						227	540	299	29	5	2	1															1103
1946						2	150	431	143	25	17	1															769
1947[a]							1	121	677	199	60	4															1062
1948[a]								2	64	108	250	87															511
1949[a]									4	236	266	366															872
1950[a]										6	16	259				1											282
1951[a]											2	31				1											34
1952[a]																6	2	1									9
1953[a]																60	5	3									68
1954[a]																292	34	4	1								331
1955[a]																288	208	27	4	2							529
1956																18	144	148	37	9	1						357
1957																1	33	292	341	61	3						731
1958																	1	36	177	166	49	1					430
1959																		2	23	212	345	40	3				625
1960																			1	35	227	317	41	2			623
1961																					13	181	194	18	3		409
1962[a]																						23	198	270	24	1	516
1963[a]																							59	306	197	4	566
1964[a]																								24	108	27	159
1965[a]																									24	133	157
1966[a]																										163	163
1967[a]																										8	8
Total	856	977	888	524	449	668	1025	920	921	580	613	749				667	427	513	584	485	638	562	495	620	356	336	

[a]Incomplete year-class.

mated an annual rate of natural mortality of 45% for whitefish in Lake Winnipeg, based on captures by fishermen of whitefish tagged in 1959. Cucin and Regier (1966) estimated a natural mortality rate of 34% for lake whitefish in southern Georgian Bay. Our rate is considerably higher, and could be expected to be so, since Cucin and Regier pointed out that catch curves based on gillnet samples always overestimate whitefish natural mortality rates, unless fish growth ceases after they become fully vulnerable to the fishing gear, or a carefully designed series of mesh sizes is used to provide a fishing gear equally efficient for all ages or sizes of fish. They further point out that the more rapidly the fish grow or the shorter the range in mesh size, the greater the error in the estimate.

In view of this, it is quite likely that our estimated value of 51% may be too high. However, Cucin and Regier noted that older whitefish in southern Georgian Bay had higher natural mortality rates than fish of intermediate ages. Therefore, our estimate of the natural mortality rate, which is based on older fish, is probably not seriously overestimated.

Virtual Population Estimates

Virtual population estimates for each year-class of whitefish were calculated as described previously. Virtual population estimates of year-class strength can be biased by changes in fishing pressure, in the availability and/or vulnerability of the fish to capture, and in their natural mortality (see the papers of Bishop (1959) and Paloheimo (1958)). Despite these limitations, virtual population estimates can provide a rough measure of year-class strength and provide information relative to the magnitude of the variation in year-class strength between strong and weak year-classes. Virtual population estimates for each year-class of whitefish are shown in Table 6, and are graphed in Fig. 6, for the 1938–50 and 1954–66 year-classes. The mean in the figure was calculated from data for the 1940–46 and 1956–61 year-classes, since these were the only ones for which complete data were available. Examination of Fig. 6 indicates that the 1938–39, 1945–47, 1949, and 1957 year-classes were strong year-classes, while the 1940–44, and 1956, 1958, and 1961 year-classes were weak. The 1959 and 1960 year-classes appear to be somewhat above average. Kennedy (1954) surmised that the 1932–39 year-classes were approximately equal in strength, the 1940–44 year-classes were about one-half as strong, and that the 1945 year-class was about as strong as the 1932–39 year-classes. The data in Fig. 6 and Table 6 suggest that the 1947 and 1949 year-classes may also have been as strong as the 1938 and 1939 year-classes. Miller (1956) suggested that the weak year-classes of the early 1940s in Lake Winnipeg and six Alberta lakes may be part of some prairie-wide phenomenon possibly associated with meteorological conditions. There is no doubt that a portion of the fluctuations in Lake Winnipeg whitefish catches can be ascribed to environmental conditions which cause fluctuating amounts of mortality of eggs and/or fry. However, the intensified fishing effort, exploitation of younger age groups, diminishing catches, and lower survival rates strongly suggest a decline in abundance caused by overfishing. Christie (1968) points out that year-class irregularity and serious stock decline in the face of heavy fishing are effects of fishing which should not be overlooked.

It might be suggested that ecological changes were responsible for the decline of the whitefish in Lake Winnipeg. Investigators have documented how unfavorable water quality has affected whitefish stocks in Lake Erie (Lawler 1965; Hartman 1972). Smith (1972) has discussed the decline of oligotrophic fish communities in the Great Lakes. There have been few limnological data collected on Lake Winnipeg which are suitable for comparative purposes. However, it is worthwhile to examine these limited data. The total dissolved solids in the north basin of Lake Winnipeg have increased by only 5 ppm from 1925–28 to 1963–64 (Rybicki 1966) or an average increase of 0.1 ppm/year. In contrast, the total dissolved solids in Lake Erie have increased at an average rate of 1.0 ppm/year from 1934–38 to 1958 (Beeton 1961, 1965). The total dissolved solids data do not suggest that a major ecological change has occurred in the north basin of Lake Winnipeg.

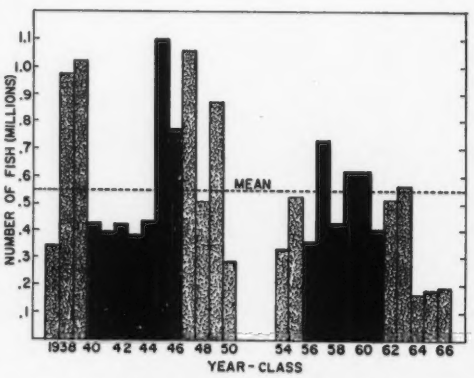


FIG. 6. Year-class strength estimates for the 1938–50 and 1954–66 year-classes of whitefish. Dark bars represent complete year-classes, while light bars represent incomplete ones.

Temperature data, although extremely limited, also suggest that no major ecological change has occurred in the north basin of Lake Winnipeg. Bajkov (1930) reported surface temperatures of 15.5 and 18.5 C at George Island on August 7 and 11, 1928, respectively. L. C. Hewson (personal communication) found a surface temperature of 16.8 C on August 8, 1954. Hewson also found a surface temperature of 17.8 C on July 25, 1954 at George Island, and Rybicki (1966) found the identical surface temperature on July 22, 1964 at George Island. It appears that water temperatures in the north basin of Lake Winnipeg have remained essentially unchanged since 1928. Rybicki (1966) compared the percentage composition of the benthic fauna population sampled by Bajkov in 1929 in the vicinity of George Island with that obtained in 1963 from stations in approximately the same general area. Assuming that the 1929 and 1963 stations were of comparable bottom types and locations, his comparison suggested that the Oligochaetes (pollution-tolerant organisms) had displaced the Amphipods (pollution-intolerant organisms) as the dominant group in the George Island area. The source of this pollution is probably from the fish station located there. Although Rybicki found Oligochaetes to be the dominant group at other stations in the north basin of Lake Winnipeg, he found that their dominance was not as marked as that in the vicinity of George Island.

The above limnological findings suggest that a slight change in total dissolved solids, no apparent change in temperature, and a major change in benthos have occurred in the north basin of Lake Winnipeg. These changes have not greatly affected whitefish production since whitefish quotas of 880,000 lb have been taken quite easily by commercial fishermen in 1971 and 1972 following the 1970 closure of Lake Winnipeg to commercial whitefish fishing. Therefore, the decline in catch of whitefish in Lake Winnipeg cannot be attributed to a major deterioration in environment such as occurred in Lake Erie. We judge that the optimum rate of fishing has been exceeded, and that the fishery itself is largely responsible for the depleted condition of the whitefish stocks in Lake Winnipeg.

Acknowledgments

We wish to record the kind cooperation of the many fishermen who permitted their catches to be sampled, and to acknowledge the effort made by various personnel of the Manitoba Department of Mines, Resources, and Environmental Management in collecting data for this study. We also thank the various scale readers and technicians who provided further development of the data. Unfortunately, it is impossible to acknowledge all the above by individual name. Special thanks are due to C. G. Prouse, who prepared all the computer programs for estimating survival rates. Appreciation is expressed to W. N. Howard and W. R. Pollard, who kindly made available portions of the manuscript reports they are currently preparing.

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Divergent Growth in Two Lake Whitefish (*Coregonus clupeaformis*) Populations[1,2]

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BIDGOOD, B. F. 1973. Divergent growth in two lake whitefish (*Coregonus clupeaformis*) populations. J. Fish. Res. Board Can. 30: 1683–1696.

Divergence in the growth pattern of Pigeon Lake and Buck Lake whitefish (*Coregonus clupeaformis*) began during the winter months in young-of-the-year fish. The growth rate of individuals in the Pigeon Lake whitefish population has declined over the past 12 years whereas individuals in the Buck Lake population have maintained a relatively stable growth pattern. These differences did not appear to be genetically controlled. Increased interspecific and intraspecific competition for the available food supply likely suppressed the growth rate of the Pigeon Lake whitefish population. Reduction of predator numbers in Pigeon Lake by the removal of habitat and increased angling pressure accelerated recruitment to both the whitefish population and other competitive feeding forage fish. A predator–prey relationship conducive to the maintenance of a faster growing lake whitefish population has been maintained in Buck Lake over the same period of time.

BIDGOOD, B. F. 1973. Divergent growth in two lake whitefish (*Coregonus clupeaformis*) populations. J. Fish. Res. Board Can. 30: 1683–1696.

Les modalités de croissance du grand corégone (*Coregonus clupeaformis*) des lacs Pigeon et Buck ont commencé à diverger pendant les mois d'hiver chez les jeunes de l'année. Le rythme de croissance des individus du lac Pigeon est allé en diminuant au cours des 12 dernières années, alors que celui des poissons du lac Buck est demeuré à peu près stable durant cette période. Les différences de croissance chez les deux populations ne semblent pas être sous le contrôle génétique. Les caractères morphométriques et limnologiques des deux lacs sont comparables. Une concurrence alimentaire inter- et intraspécifique accrue est probablement responsable du ralentissement de la croissance du grand corégone dans le lac Pigeon. La réduction du nombre de prédateurs, dans le lac Pigeon, par élimination d'habitats et pêche sportive plus intense a favorisé le recrutement tant du grand corégone que des autres poissons fourrage compétiteurs. Une relation prédateur–proie favorisant un rythme de croissance plus rapide du grand corégone a été maintenue dans le lac Buck au cours de la même période.

Received December 13, 1972

PIGEON and Buck lakes have been commercially fished with gillnets for lake whitefish, *Coregonus clupeaformis*, since about 1920[3]. Both these eutrophic, Alberta lakes have been fished with 140-mm stretched mesh nets from 1942 to 1967. In 1968, the legal mesh size for the Pigeon Lake commercial fishery was reduced to 114-mm mesh to facilitate economical harvest of the commercial quota. The following year, a further reduction in the legal mesh size to 89 mm was deemed necessary. In 1971, the Freshwater Fish Marketing Board would not accept Pigeon Lake whitefish because they were not of marketable size. Since 1942, the commercial harvest of whitefish from Buck Lake has remained relatively stable with continuous use of 140-mm stretched mesh gillnets.

The decline in the growth rate of the Pigeon Lake whitefish and the sustained growth pattern of the Buck Lake whitefish provides an opportunity to investigate factors controlling the growth rate of lake whitefish in two similar lakes by studying and comparing the ecology of these populations of fish. A thesis is presented on the cause of the

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology, The University of Alberta, 1972.

[2]Presented at the 26th Annual Meeting of the Canadian Committee on Freshwater Fisheries Research, Halifax, January 3–4, 1973 at the session, "Over-exploitation of fish stocks — a view in 1973," convener J. J. Keleher.

[3]Abstracts from Seminar Papers, Canada Department of Fisheries.

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decline in the growth rate of the Pigeon Lake population. Field studies and experiments, initiated in 1968 and terminated in 1972, compared the growth rates and feeding habits of these two populations of whitefish in their respective ecosystems.

Materials and Methods

Physico-Chemical Measurements

Surficial lake sediments in Pigeon and Buck lakes were sampled with a 152-mm weighted Petersen dredge, by visual observations from the surface and by scuba diving. Since the clearing of aquatic plants accompanied the construction of cottages on shoreline lots, the locations of rooted aquatic plants on both lakes were documented in July of the same year and the increase in the number of cottages on shoreline lots on Pigeon and Buck lakes was enumerated from aerial photographs taken in 1950 and again from photographs taken in 1966.

Limnological stations were at 8.2 m depth in Pigeon Lake and 9.1 m depth in Buck Lake over surficial lake sediments of silt (Fig. 1). Sampling was undertaken on both lakes within 3 hr of the same day. Water temperatures were recorded at 0.6-m intervals from the surface to the bottom. Dissolved oxygen concentrations determined with a Hach kit were taken at the surface, midwater and 76 mm from the bottom of each lake on the same day at the two designated limnological stations.

Sampling Procedures

Benthos — The bottom fauna in Pigeon and Buck lakes was sampled with a 152-mm Ekman dredge in each of three surficial lake sediments in each lake within 2 consecutive days. Three samples taken in each lake sediment of silt, sand, and rubble were washed in a screen bottom pail of 12 meshes/cm.

Plankton — Plankton were sampled at each limnological station on both lakes (Fig. 1) within the same 2 days of each month. Three water samples (76 mm from the bottom, midwater, and surface) collected with a modified Van Dorn water bottle that trapped 3.3 liters of water, were strained through a 12-cm Wisconsin plankton net with 20 mesh nylon bolting cloth in both the upper net and straining bucket. Each sample was preserved in 70% ethanol and the zooplankton were counted and identified in the laboratory.

Fish — Samples of fish were collected from each lake with gillnets, both monofilament and braided nylon, of varying mesh sizes; beach seines; brail net casts; meter trawl tows, and a 4.9-m outboard trawl with a 1.3-cm mesh liner in the cod end. Length, weight, sex, and maturity were recorded for each whitefish sampled and scales were removed for age determination. Stomachs were removed and the contribution of the various food items to the relative fullness was estimated by a technique described by Thompson (1959). Samples of young-of-the-year (y-o-y) Pigeon and Buck Lake whitefish hatched from eggs reared in the laboratory under the same temperature regimes and for the same duration of time were placed in four 38-liter aquaria the same day of hatching, reared under the same ambient temperature (12.5–15.0 C) with the same daily photoperiod and offered the same quantity of brine shrimp, *Artemia salina*, per fish and measured following two rearing periods. Further descriptions of the methods are presented by Bidgood (1972). Student's "*t*" test for unpaired observations and unequal variances was employed in the statistical comparison of any two means of samples with equal or unequal observations (Steel and Torrie 1960).

Field Study Area

Both lakes are located on Western Alberta Plains within the same major drainage basin and at the same latitude (Fig. 1). The bedrock is sandstone, shale, and coal while the surficial deposits of the area are ground moraine, hummocky moraine, and till. Although Pigeon Lake is about four times the size of Buck Lake, the morphometry is comparable:

Measurement	Lake	
	Pigeon	Buck
Surface area (*ha*)	9591	2486
Elevation (*m*)	849.5	881.5
Maximum length (*km*)	16.5	10.2
Maximum width (*km*)	8.1	4.7
Maximum depth (*m*)	9.1	12.2
Mean depth (*m*)	6.3	6.2
Shoreline length (*km*)	41.5	35.1
Shore development ratio	1.19	1.98

No surface discharge and only intermittent drainage into Pigeon Lake occurred during the course of these studies. Surface discharge in Buck Lake is north through Buck Lake Creek to the North Saskatchewan River. Three permanent drainage systems discharge water into Buck Lake.

Oxygen–Temperature Regimes

The temperature regime, period of open water and ice cover, and dissolved oxygen concentrations in Pigeon and Buck lakes compared in the same year were similar (Fig. 2). Buck Lake, being smaller was less subject to wind and acquired ice cover from 5 to 7 days ahead of Pigeon Lake. Conversely, because of the greater discharge of water into the lake, ice cover disappeared from Buck Lake from 5 to 7 days ahead of Pigeon Lake. Both lakes were continuously mixed by summer winds and did not exhibit chemical or temperature stratification. Oxygen concentrations in both lakes under ice cover would not limit the distribution of the fishes.

Species Composition

Nine and 10 species of fish were captured in Pigeon (P) and Buck (B) lakes, respectively:

Species	Lake (P)	Lake (B)
Lake whitefish, *Coregonus clupeaformis*	X	X
White sucker, *Catostomus commersoni*	X	X
Burbot, *Lota lota*	X	X
Yellow perch, *Perca flavescens*	X	X
Northern pike, *Esox lucius*	X	X
Spottail shiner, *Notropis hudsonius*	X	X
Emerald shiner, *Notropis atherinoides*	X	–
Trout-perch, *Percopsis omiscomaycus*	X	X
Walleye, *Stizostedion vitreum vitreum*	–	X
Iowa darter, *Etheostoma exile*	X	X
Brook stickleback, *Culaea inconstans*	–	X

Walleyes were last recorded in the Pigeon Lake commercial fishery in 1963–64 whereas a total of 945 walleyes were captured in gillnets set purposely to avoid walleyes and capture whitefish in Buck Lake. A total of 17 northern pike were captured in Pigeon Lake as opposed to 431 captured in Buck Lake throughout the 5-year sampling period even though a concerted effort was made to capture pike in Pigeon Lake and to avoid pike in Buck Lake. Other species of fish were noticeably more abundant in Pigeon Lake than in Buck Lake. Only three trout-perch were captured in Buck Lake during the course of these studies, whereas an overnight set of 30 m of 1.91-cm monofilament gillnet in Pigeon Lake would yield over 2000 trout-perch.

The density and species of rooted aquatic plants in Pigeon Lake was less and fewer than that observed in Buck Lake (Fig. 3) in July, 1969 and occurred in both lakes up to a depth of 2 m of water. The 3-m contour occupies about 18 and 28% of the total area of Pigeon and Buck lakes, respectively. Only about 23% of the area within the 3-m contour in Pigeon Lake has rooted aquatic plants in contrast to 83% in Buck Lake.

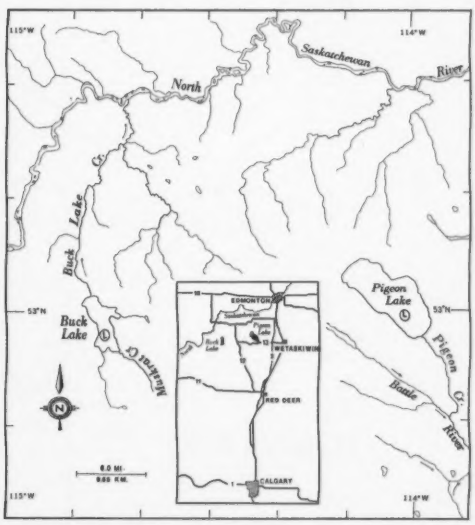

FIG. 1. Location of Pigeon Lake relative to Buck Lake in the North Saskatchewan River drainage system. The locations of the limnological stations (L) are designated. The insert locates the study lakes relative to major Alberta cities and highways.

Whitefish Growth

Young-of-the-Year

A divergence in the growth pattern of Pigeon and Buck Lake whitefish occurred before the fish formed their first annulus. Young-of-the-year whitefish from Pigeon and Buck lakes hatched and reared under the same laboratory conditions had similar growth rates (Table 1). Divergence in the growth rate of Pigeon and Buck Lake lake whitefish began in y-o-y fish during their first winter (Table 2). The Buck Lake population of y-o-y fish had a similar growth rate to that of the Pigeon Lake fish during the summer months but had a faster growth rate during the winter months. Gangs of 1.9-cm, 2.5-cm, and 3.8-cm stretched mesh monofilament gillnets were set in both lakes to collect samples of y-o-y fish through the ice and at spring break-up. Buck Lake sampling preceded that of Pigeon Lake by 1 week in all cases. The small sample size (3) from Buck Lake in December prevented valid statistical comparison of the mean size of samples from the two lakes even though the means differed by 10 units, the same differences found significant in comparisons of larger samples captured in different sampling periods. In early June, all yearling fish were captured in the 3.8-cm nets in Buck Lake and all yearling fish were captured in the 2.5-cm nets in Pigeon Lake. Yearling fish appeared relatively more abundant in Pigeon Lake than Buck Lake since over nine times the fishing effort in Buck Lake was required to capture 38 yearling fish than that of Pigeon Lake to capture 44 yearling fish.

Juveniles and Adults

On comparing the growth rates of samples of lake whitefish captured in Buck Lake in the third

week of September and Pigeon Lake in the fourth week in 2 consecutive years, all age-classes in the Buck Lake samples were larger than that of the same six age-classes from Pigeon Lake (Table 3.) Growth occurred between years in both populations but the rates of growth differed. Even though the sampling period and gear was standardized in these 2 successive years of sampling, differences between the growth of individual age-classes between years was obvious. A reduction in the growth rate

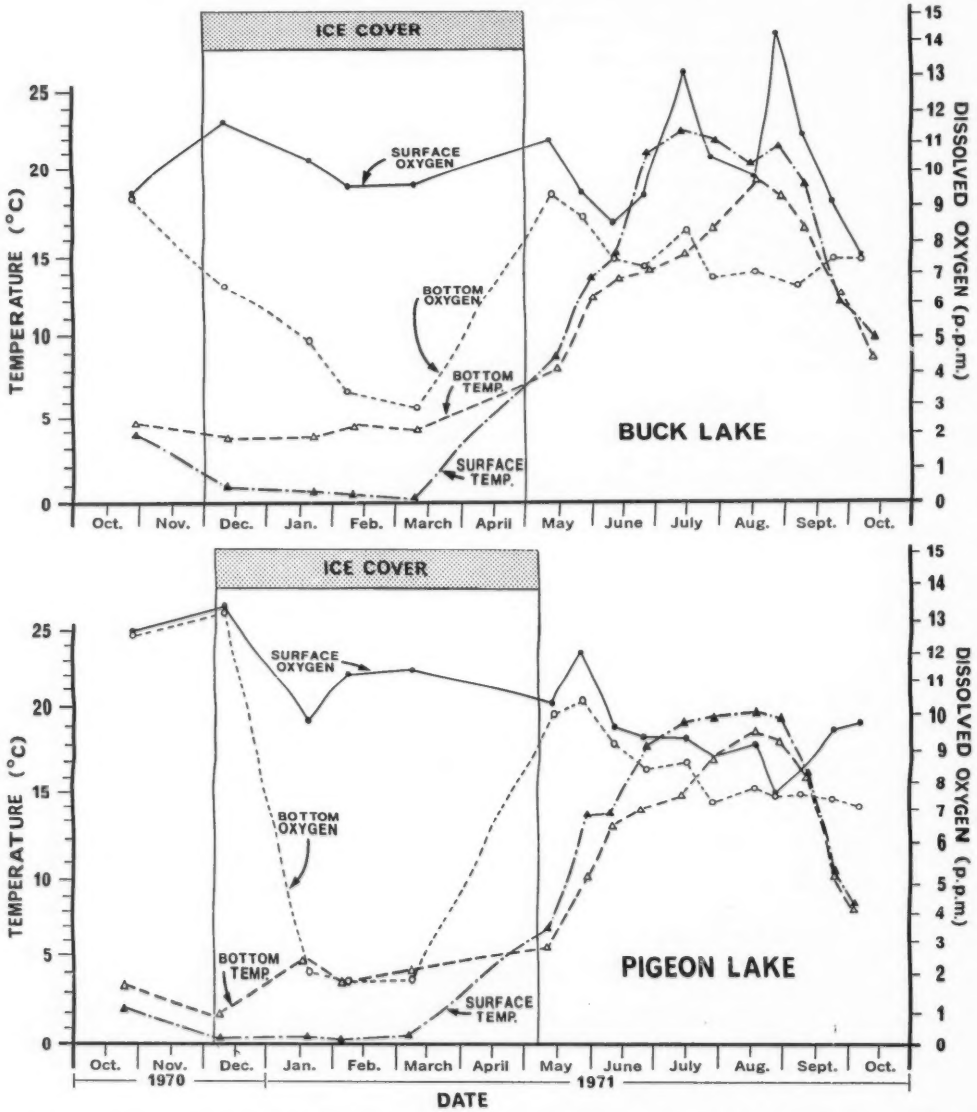

FIG. 2. Water temperature and dissolved oxygen concentrations at the surface and bottom of Pigeon and Buck Lake. The period of ice cover on both lakes is indicated.

of individuals in the Pigeon Lake population has occurred since 1954 and the Buck Lake whitefish population had a faster growth rate than the Pigeon Lake population in both 1954 and 1970 (Table 4). On comparing the growth data from the present studies with the weighted means of four age-classes of fish captured in the fall of 1951 to 1954 inclusive, the four age-classes of fish in 1954 were larger than the same age-classes in 1970. The collapse of the 14-cm gillnet commercial fishery in 1968 also documents the reduction in the growth rate of the Pigeon Lake population of whitefish since 1954.

Factors Affecting Growth

Population Characteristics

From a sample of 4455 lake whitefish from Pigeon Lake and 3121 from Buck Lake, the sex ratio of lake whitefish from both lakes was proportionate. In spite of the differential growth rates previously noted between these two populations, 15–20% of the samples of both sexes collected in the fall having three completed annuli were mature, 96–97% of the samples with four completed annuli were mature and all individuals in the samples with five completed annuli were mature. No individuals with two completed annuli were found mature in either lake. Mature fish in both lakes spawned every year since no retention of eggs was observed and no maturing fish (IV + and older) were noted in the whitefish samples collected from January to March in both lakes. Annulus formation began in late April coincident with a rise in lake water temperature and an increase in the mobility and feeding activity of the whitefish populations. No difference in the length of the sex components of a given age class of whitefish was observed when the means of these partitioned groups were statistically compared.

Pigeon Lake had a mature population of lake whitefish (IV+ and older) that was about three times the number per unit area as that of Buck Lake. Mature lake whitefish were about four times more active during the open water period than under ice cover. The mean catch per unit effort of mature fish, defined as the number of mature

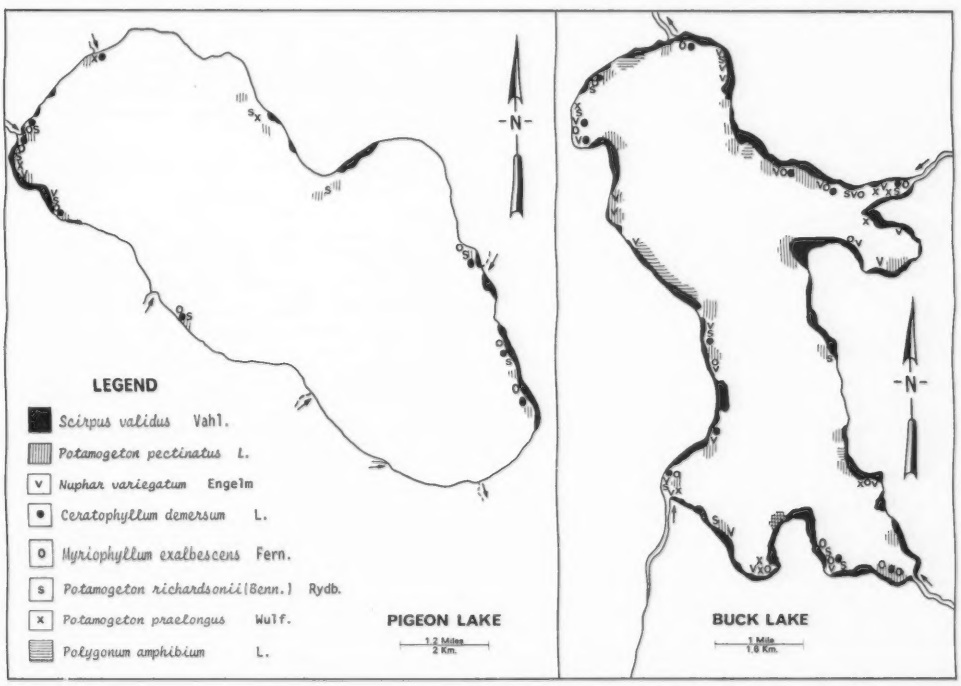

FIG. 3. Location and concentration of seven species of rooted, perennial aquatic plants in the littoral zone of Pigeon Lake and eight species in Buck Lake.

fish (IV+ and older) captured in a 24-hr set of 91 m of 210/3 nylon gillnet 2.4 m in depth, was 162.7 fish in Pigeon Lake and 52.8 fish in Buck Lake during the open water sampling periods. Under ice cover, the mean catch per unit effort in Pigeon Lake was 38.7 fish as opposed to 13.0 mature fish in Buck Lake.

Even though the growth rate in Pigeon Lake was much slower than that in Buck Lake (Table 3) the age at maturity was similar. Both Budd and Cucin (1962) in studies on Lake Huron whitefish and Hart (1930) in studies on Lake Ontario whitefish reported that these populations matured in their fifth year of life (four completed annuli) whereas Cucin and Regier (1966) reported that male lake whitefish with four or more completed annuli and females with five or more completed annuli in Georgian Bay were mature. Most of Pigeon and Buck Lake male and female fish with four completed annuli were mature and all fish of both sexes in the samples with five completed annuli were mature. The studies of Kennedy (1953) and the present studies found both male and female fish maturing at the same age and size. Contrary to the findings of Miller (1947) on Pigeon Lake and consistent with those of Rybicki and Doan (1966) on Lake Winnipeg, the differential growth rates of the Pigeon and Buck Lake whitefish populations in the present studies did not alter the age of maturity of these two populations. The sex ratio of all fish sampled from collections both on and off the known spawning beds approached a 50:50 ratio in both lakes. These findings are consistent with those of Miller (1956) in Pigeon Lake and Kennedy (1943) in Lake Opeongo, Algonquin Park, Ont.

TABLE 1. Comparisons of the mean+ SE total lengths (mm) of samples of lake whitefish from Pigeon and Buck lakes hatched from eggs, reared under the same laboratory conditions, and measured at two periods from the hatching date. Number of observations is 30 in all. Nonsignificant$_{.05}$ comparisons ($\simeq$) of samples are indicated.

Pigeon Lake		Buck Lake	
57 days	60–63 days	57 days	60–63 days
31.53±0.32	31.50±0.33	31.33±0.50	32.00±0.27
$\simeq$	$\simeq$	$\simeq$	

TABLE 2. Comparisons of the mean ± SE fork lengths (mm) of trawl caught (T) and monofilament caught (M) samples of y-o-y and yearling lake whitefish from Pigeon and Buck lakes. Number of observations is in parentheses. Highly significant$_{.01}$ greater ($>>$) and nonsignificant$_{.05}$ ($\simeq$) comparisons of means are indicated.

Sampling gear	Age	Pigeon Lake Date	Pigeon Lake $\bar{x}$		Buck Lake $\bar{x}$	Buck Lake Date
T	0+	Aug. 19, 1971	89.61±0.70 (36)	$\simeq$	91.15±0.75 (41)	Aug. 18, 1971
				∧ ∧		
T	0+	Sept. 8, 1971	103.00±1.11 (7)	$\simeq$	102.70±0.54 (71)	Sept. 1, 1971
				∧ ∧		
M	0+	Dec. 17, 1971	119.46±0.87 (48)	?	129.00±6.08 (3)	Dec. 30, 1971
			∧ ∧		∧ ∧	
M	1+	June 6,1972	131.79±1.11 (44)	<<	143.03±1.15 (38)	May 31, 1972

TABLE 3. Comparisons of the mean+ SE fork lengths (mm) of six age-classes of Pigeon and Buck Lake whitefish sampled in the latter 2 weeks of September of 1971 and 1972. Number of observations is 30 in all, except for Pigeon/71, two annuli where n = 27. Highly significantly$_{.01}$ greater (>>), significantly$_{.05}$ greater (>) and nonsignificant ($\simeq$) comparisons of means are indicated.

	Number annuli					
Lake/Year	I	II	III	IV	V	VI
Pigeon/70	189.73±1.79	248.80±2.67	313.20±4.23	348.60±2.65	358.07±2.87	367.77±2.73
	<<	<<	<<	<	<	
	V V	≷	∧	≷	∧ ∧	∧ ∧
Pigeon/71	183.40±1.56	244.70±5.98	328.27±4.85	357.27±4.00	373.17±2.68	379.00±2.38
	<<	<<	<<	<<	≃	
	∧ ∧	∧ ∧	∧ ∧	∧ ∧	∧ ∧	∧ ∧
Buck/70	213.57±3.73	345.00±7.14	388.43±2.88	450.53±6.25	478.50±6.05	475.57±4.72
	<<	<<	<<	<<	≃	
	∧ ∧	V V	≷	≷	≷	∧ ∧
Buck/71	232.67±4.30	313.20±3.74	385.07±2.67	441.40±2.74	483.03±4.93	502.30±4.16
	<<	<<	<<	<<	<<	

TABLE 4. Comparisons of the mean+ SE fork length (mm) of four age classes of whitefish from 1954 Pigeon Lake samples with the 1970 and 1971 samples collected in the fall of each year from Pigeon and Buck Lake. Number of observations for Pigeon (Miller 1956) in parentheses, for all others n = 30. Highly significantly$_{.01}$ greater (>>), comparisons of means are indicated.

	Number annuli			
Lake	III	IV	V	VI
Pigeon (Miller 1956)	345.93±1.17 (47)	372.91±0.48 (145)	391.42±0.29 (338)	415.56±0.41 (127)
	V V	V V	V V	V V
Pigeon (1970)	313.20±4.23	348.60±2.65	358.07±2.87	367.77±2.73
	∧ ∧	∧ ∧	∧ ∧	∧ ∧
Buck (1970)	388.43±2.88	450.53±6.25	478.50±6.05	475.57±4.72

Habitat Manipulation and Selective Angling

Large urban centers such as Edmonton, Wetaskiwin, and Red Deer are in close proximity to Pigeon Lake (Fig. 1). A base paved road was completed from highway number two to Pigeon Lake in 1957 allowing convenient, public access to Pigeon Lake. Between 1950 and 1966, the number of shoreline cottages on Pigeon Lake increased from 250 to 845 units, whereas the number of shoreline cottages on Buck Lake increased from eight to 29 over the same period of time. Buck Lake has only about 5 miles less shoreline than Pigeon Lake. The extent of cottage development on shoreline lots was positively correlated with littoral zones devoid of rooted aquatic plants. Removal of rooted aquatic plants with aquatic herbicides and/or by mechanical means was observed in both lakes during the course of these studies.

The optimum spawning habitat for northern pike is a bottom with a dense mat of short vegetation. This vegetation acts as both a stimulus to northern pike spawning and serves as a substrate for the attachment of spawned and fertilized eggs. The hatching y-o-y pike attach themselves by means of a sticky secretion of the head to plants and debris. The progeny remain in this belt of dense vegetation in shallow water for the available food supply and protection from predators. Outside this belt of dense vegetation young pike would experience increased mortality (Fabricus and Gustafson 1958; Clark 1950; Forney 1968).

Walleye and northern pike populations in Pigeon Lake were reduced by the increased angling pressure that accompanied the increased summer cottage development on the lake. Angling in the summer was selective for the predator species, walleyes and northern pike, and nonselective for the prey species, lake whitefish and other forage fish. Lake whitefish were not available to the angler in these lakes during the open water period. Angling pressure for these predators on Buck Lake with limited cottage development would have been less than that of Pigeon Lake. Angling does not exist during the open water period on Pigeon Lake at the present time whereas angling for walleye and northern pike was evident during the open water period on Buck Lake.

Pigeon Lake with suitable, adequate spawning habitat for lake whitefish likely had adequate spawning habitat for walleyes. Since no walleyes were captured in any of the sampling gear employed in the 5 years of study on Pigeon Lake and walleyes were last reported in the commercial harvest from Pigeon Lake in 1963–64, this species has been reduced in numbers or eliminated from Pigeon Lake. Buck Lake walleyes were documented as spawning in the lake in the spring on the same surficial lake sediments of boulders, sand, and gravel, as lake whitefish utilized for spawning in the fall. A reduction in the size of the northern pike population in Pigeon Lake by the reduction in the spawning and recruitment habitat for this species likely upset the ecological balance between predator and prey previously established in this lake. With a reduction in the numbers of the one predator, northern pike, the numbers of lake whitefish surviving to maturity likely increased and the recruitment to this population increased yearly. Pigeon Lake lake whitefish fed on lake whitefish and burbot eggs (Fig. 4) and probably fed on walleye eggs when available in the spring. They also fed on yellow perch y-o-y up to a total length of 27 mm. Young-of-the-year walleyes were likely consumed by whitefish when present in Pigeon Lake. With a yearly increase in the recruitment to the lake whitefish population in Pigeon Lake and the resulting increase in intraspecific competition for food, the predation on walleye eggs and walleye y-o-y likely increased. The prey species probably contributed to the decline of this predator species in this ecosystem.

Feeding Habits

Yearling and older lake whitefish in Pigeon Lake fed on similar food items throughout the year and yearling and older Buck Lake whitefish had comparable feeding habits. Samples of stomachs from Pigeon Lake whitefish partitioned into 25-mm size-class ranges that ranged from 101 to 500 mm in fork length, revealed that the percent contribution of food items to the stomach contents of the partitioned samples were similar. Stomach samples from Buck Lake whitefish partitioned into 25-mm fork length size-class ranges that ranged from 201 to 700 mm, revealed that the percent contribution of food items to the stomach contents of the partitioned samples were also similar.

The percent contribution of food items to the diet of Pigeon Lake whitefish differed from that of Buck Lake fish when examined over a 12-month period (Fig. 4). The relatively uniform feeding habits of the Buck Lake population contrasted to the variability in the feeding habits of the Pigeon Lake population when examined over the same period of time and the contribution of planktonic food items, Cladocera and Copepoda, to the diet of Pigeon Lake whitefish differed from that of Buck Lake whitefish when examined over a 12-month period. Lake whitefish eggs were a major contributor to the diet of Pigeon Lake whitefish from October to January and burbot eggs were a major food item of the Pigeon Lake whitefish from March to May. Lake whitefish eggs made only a

small contribution to the diet of Buck Lake whitefish in December and January. Amphipoda and Pelecypoda were more prominent in the diet of Buck Lake fish throughout the year. Tendipedidae were prominent food items in the diet of Buck Lake whitefish throughout the year but were only a major contributor to the diet of Pigeon Lake whitefish during the summer months. Zooplankton was a major food item of the Pigeon Lake whitefish over the winter months (December–March) whereas bottom fauna was the major food item of Buck Lake fish throughout the winter months. Zooplankton was a more prominent food item in the diet of Buck Lake fish in the open water period (May–October) than that of the Pigeon Lake whitefish.

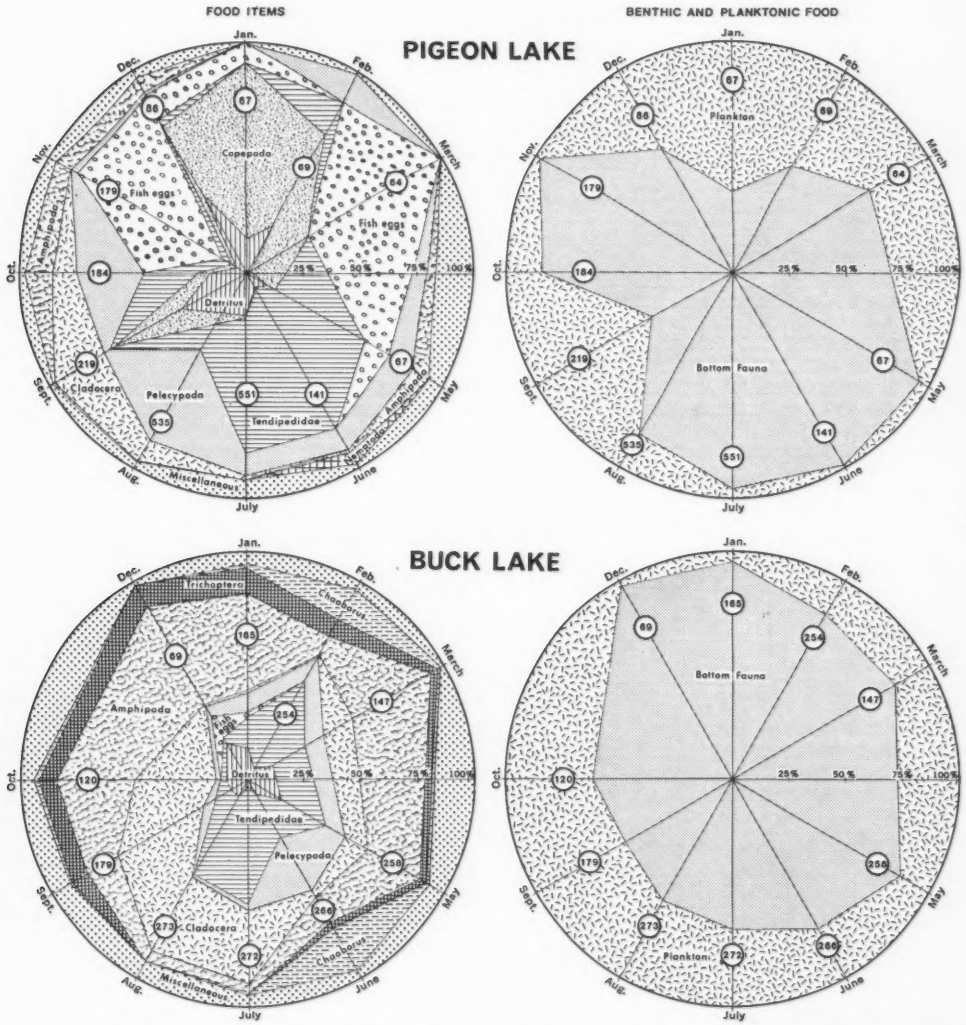

FIG. 4. Percent contribution of food items and benthic and planktonic food to the diet of Pigeon and Buck Lake yearling and older lake whitefish over a 12-month period. The monthly sample size is presented on the radii.

The feeding habits of y-o-y lake whitefish populations from Pigeon Lake paralleled that of Buck Lake during their first year of life. Both Buck Lake and Pigeon Lake y-o-y fed on zooplankton (Cladocera and Copepoda) and aerial insects during August and September and continued feeding on zooplankton during their first winter of life. After the ice left these lakes in the spring, the yearling fish in both lakes began feeding on bottom fauna.

The availability of food played a more important role in the feeding habits of Pigeon and Buck Lake whitefish populations than did selection for food items. Even though the standing crop of bottom fauna in Buck Lake was greater than that of Pigeon Lake from August to October (Fig. 5), the Buck Lake population of yearling and older lake whitefish fed on plankton to a greater extent during this period than did the Pigeon Lake population of yearling and older fish (Fig. 4). At this time of year, the number of Cladocera increased in Buck Lake but not in Pigeon Lake (Fig. 6). These Daphnia were carrying eggs at this time of year and were likely conspicuous to the feeding Buck Lake whitefish. Watson (1963) in studies on Heming Lake, Manitoba, Hart (1930) in studies on Bay of Quinte, Lake Ontario, and Quadri (1961) in studies on Lac La Ronge, Saskatchewan also concluded that lake whitefish were opportunists with regard to feeding habits.

Predators and Competitors

The walleye in Buck Lake were predators of y-o-y and yearling whitefish as well as being predators of spottail shiners, burbot, yellow perch, northern pike, and smaller walleyes. Northern pike were also predators of lake whitefish in Buck Lake, the size of the prey depended on the size of the predator. Yellow perch, burbot, northern pike, and spottail shiners were also prey species of northern pike in Buck Lake. Burbot in both lakes were predators of lake whitefish (o+ and 1+), yellow perch, sticklebacks, and burbot. In a sample of 72 northern pike stomachs, 35 stomachs contained food and 22 of the 35 contained fish. Lake whitefish were identified in five of these 22 stomachs, spottail shiners in two, burbot in four, yellow perch in eight, northern pike in three, walleye in one, trout-perch in one, and unidentified fish in two stomachs. In a sample of 450 walleyes, 67 of the 205 stomachs with food contained fish. Lake whitefish were present in 21 stomachs, spottail shiners in eight, burbot in 15, yellow perch in 14, northern pike in one, and unidentified fish in 32 stomachs. In a sample of 105 burbot, 33 stomachs contained food and 32 contained fish. Lake whitefish was identified in one stomach, spottail shiners in two, burbot in two, yellow perch in four, sticklebacks in one, and unidentified fish in 13 stomachs.

Competitors of lake whitefish for bottom fauna in Pigeon Lake were trout-perch, emerald shiners, spottail shiners, yellow perch, white suckers, and burbot. With the exception of emerald shiners and the addition of brook sticklebacks, the same competitors for bottom fauna were present in Buck Lake.

Lake Productivity

Even though Pigeon Lake water had a specific conductance of 216 μmho and Buck Lake 204 μmho at 18 C and both lakes had similar mean depths, the standing crop of bottom fauna in three surficial lake sediments was higher in Buck Lake than in Pigeon Lake (Fig. 5) and the standing crop of macrozooplankton in Buck Lake was higher than that of Pigeon Lake (Fig. 6).

Hyalella azteca were numerous in the July silt sample from Pigeon Lake and accounted for the higher total weight biomass in Pigeon Lake over that of Buck Lake for that month. The amount of bottom fauna available to bottom feeding fish is higher in Buck Lake than in Pigeon Lake during the winter months. *Gammarus l. lacustris* was noticeably absent from the Pigeon Lake samples but present, although in lower abundance than *H. azteca*, in the Buck Lake samples. With the exception of two sampling periods, the standing crop of Copepoda in Pigeon Lake was lower than that of Buck Lake. The standing crop of Cladocera was higher in Buck Lake than Pigeon Lake in all but one of the 13 sampling periods. A pulse in the standing crop of Cladocera occurred in Buck Lake beginning in August, peaking in October and declining in November and again in December. No such pulse was evident in Pigeon Lake during the same period of time. *Cyclops bicuspidatus thomasi* was the most common copepod noted in the samples from both lakes. *Diaptomus sp.* was less common but found in the samples from both lakes. *Daphnia similis* was the most common species of Cladocera noted in the samples from both lakes. *Leptodora kindti* was noted as present in both these eutrophic lakes.

Conclusion

Although Pigeon and Buck lakes are located in the same major drainage basin and at the same latitude, have similar morphometry, temperature, and dissolved oxygen regimes, they support lake whitefish populations with dissimilar growth rates.

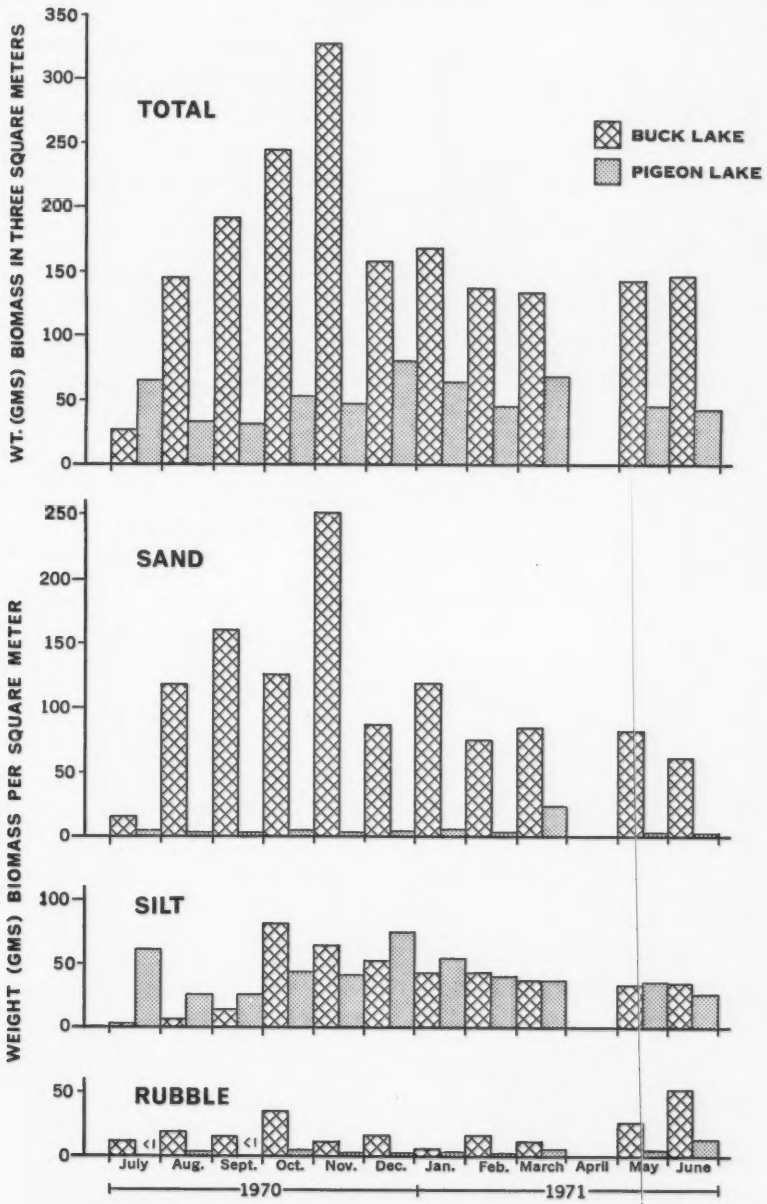

FIG. 5. Mean weight biomass of the standing crop of invertebrates in three samples of an Ekman dredge in each of three surficial lake sediments. The total biomass of invertebrates in the three surficial lake sediments is presented.

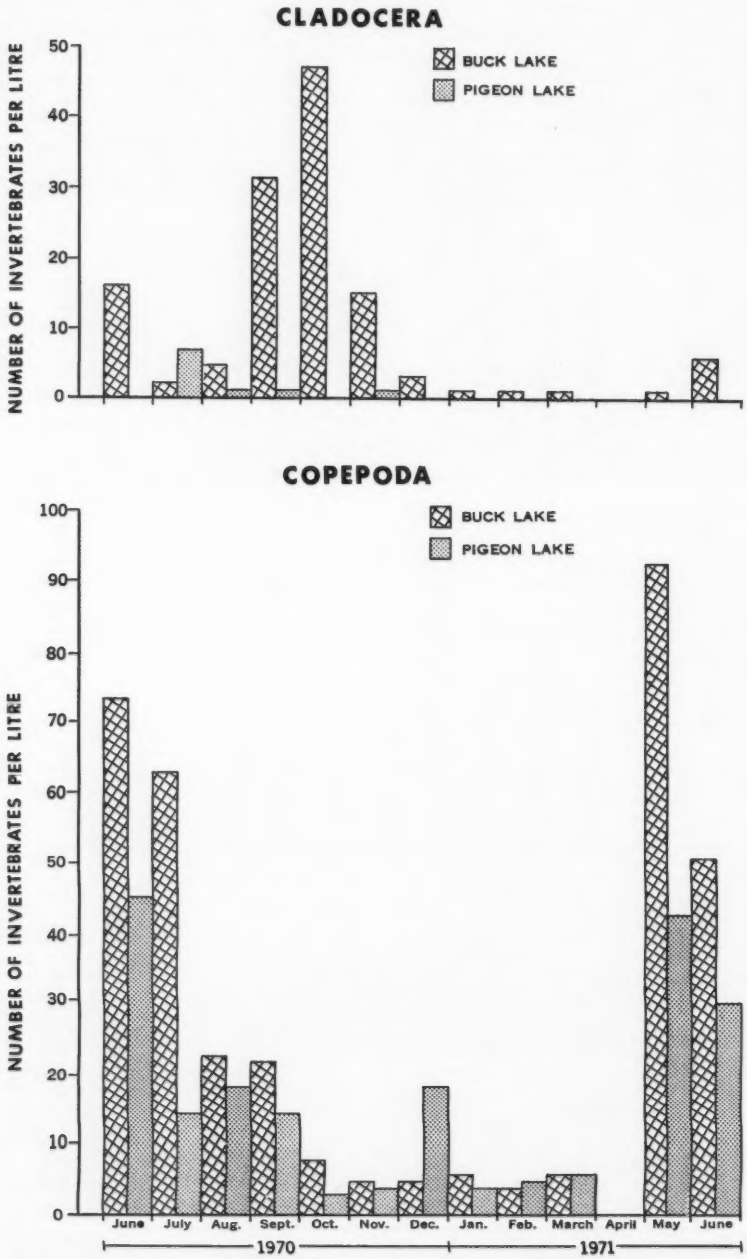

FIG. 6. Mean number of Cladocera and Copepoda per unit volume in water samples collected at three depth strata in Pigeon and Buck lakes.

The Buck Lake population of this species has sustained a similar growth pattern since 1954 whereas the growth pattern of the Pigeon Lake population has declined over the same period of time. Pigeon Lake supports about three times the number of mature individuals per unit areas as Buck Lake.

Divergence in growth between Pigeon Lake and Buck Lake whitefish populations began during the winter months of their first year of life. The population of lake whitefish in both Pigeon and Buck lakes had similar growth rates throughout the first summer but diverged in growth during the first winter. During the first summer and the first winter, y-o-y lake whitefish in both lakes fed predominantly on zooplankton. In May of the following spring both populations of yearling fish began feeding on bottom fauna. Although Buck Lake y-o-y fish were plankton feeding during their first winter, yearling and older fish were predominantly bottom feeding during the winter months. Pigeon Lake y-o-y whitefish were also plankton feeding during the first winter but were in direct competition for food with the predominantly plankton feeding yearling and older Pigeon Lake whitefish. Buck Lake had a higher standing crop of plankton throughout the winter months than Pigeon Lake. Cropping of the plankton by the total Pigeon Lake whitefish population and cropping of the plankton by only the y-o-y Buck Lake whitefish population could have accounted for the lower standing crop of plankton in Pigeon Lake.

Pigeon Lake had a lower standing crop of bottom fauna than Buck Lake throughout the year. Both the increased number of lake whitefish and the increased number of other competitors for bottom fauna likely had caused an increased cropping of bottom fauna in Pigeon Lake in excess of that of Buck Lake. Increased competition for bottom fauna in Pigeon Lake in the winter months likely forced the total Pigeon Lake population onto a diet consisting primarily of zooplankton. Reduced interspecific and intraspecific competition for bottom fauna by predators in Buck Lake allowed the surviving lake whitefish population to exist on primarily a bottom fauna diet throughout the winter months. Not only do the y-o-y Pigeon Lake whitefish fall behind the growth rate of the Buck Lake y-o-y whitefish in the winter but the growth rate of the winter, plankton feeding yearling, and older Pigeon Lake whitefish is likely lower than that of the bottom fauna feeding yearling and older Buck Lake fish. Yearling and older Pigeon Lake whitefish could also have a reduced growth rate during the summer months over that of the Buck Lake fish because of the lower standing crop of bottom fauna available to the larger number of Pigeon Lake fish during this accelerated growth period.

Manipulation of habitat by man can change the ecological balance previously established in a given ecosystem and detrimentally alter the form of the harvested species of fish. Removal of predator habitat accompanied by a reduction in the number of predators in Pigeon Lake reduced the size and increased the numbers of lake whitefish and other competitive prey species. The total biomass of fish in Pigeon Lake is probably still relatively constant but the physical size and numbers of the aggregate fish populations contributing to this biomass have been altered.

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Temperatures Selected Seasonally by Four Fishes from Western Lake Erie

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BARANS, C. A., AND R. A. TUBB. 1973. Temperatures selected seasonally by four fishes from western Lake Erie. J. Fish. Res. Board Can. 30: 1697–1703.

When four species of fish were taken from western Lake Erie in each of four seasons and held usually for less than 7 days at ambient lake temperatures, the temperatures they selected during 2–3 days in a horizontal temperature gradient differed seasonally. The differences were largely attributable to the conditions at which the fish had been acclimatized in the lake, and were modified by acclimation during 2–3 days in the gradient.

The selected temperatures provided insights into the temperatures that might be selected by these species each season if the lake basin or other waters with similar seasonal ambient temperatures were subjected to thermal discharges. Temperatures selected were above ambient lake temperatures except for emerald shiners (*Notropis atherinoides*) in summer and fall. In general, white bass (*Morone chrysops*) and smallmouth bass (*Micropterus dolomieui*) selected a high range in temperatures throughout the year (18–30 C and 18–31 C, respectively), yellow perch (*Perca flavescens*) an intermediate range (10–29 C) and emerald shiners the lowest range (6–23 C). Three of the species were distributed within a relatively precise temperature range in the summer and within a larger range during other seasons; emerald shiners selected a narrow range during all seasons. A fairly stable temperature preference was usually reached within several hours in summer, but the temperatures selected by three species generally increased with time in the gradient during the other seasons; emerald shiners selected constant temperatures in all seasons. Temperatures selected by young and adults differed mainly in yellow perch and emerald shiners in summer and winter, when the lake temperatures fluctuated least.

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Quatre espèces de poissons capturés dans la partie ouest du lac Erié à chacune des quatre saisons et ordinairement maintenus pendant moins de 7 jours aux températures ambiantes du lac choisissent des températures variables selon la saison, lorsque placés pendant 2 ou 3 jours dans un gradient horizontal de températures. Les différences sont attribuables en grande partie aux conditions auxquelles les poissons ont été acclimatés dans le lac, et sont modifiées par l'acclimatation de 2 ou 3 jours dans le gradient.

Ces constatations nous donnent une idée des températures que ces espèces peuvent choisir à chaque saison, si le bassin du lac ou autres plans d'eau de températures ambiantes semblables reçoivent des déversements d'eau chaude. Les espèces choisissent des températures supérieures à celles qui prévalent dans le lac, sauf le méné émeraude (*Notropis atherinoides*) en été et en automne. En général, le bar blanc (*Morone chrysops*) et l'achigan à petite bouche (*Micropterus dolomieui*) choisissent une gamme de températures élevées pendant toute l'année (18–30 C et 18–31 C respectivement), la perchaude (*Perca flavescens*) un intervalle intermédiaire (10–29 C) et le méné émeraude les plus basses températures (6–23 C). Trois des quatre espèces sont réparties dans une eau de température relativement bien définie en été et dans une gamme plus étendue aux autres saisons; le méné émeraude choisit une gamme étroite en toute saison. En été, le choix d'une température relativement constante se fait en dedans de plusieurs heures, mais les températures choisies par trois espèces dans le gradient augmentent généralement avec le temps aux autres saisons; le méné émeraude choisit une température constante en toute saison. Les températures choisies par les jeunes et les adultes diffèrent surtout chez la

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perchaude et le méné émeraude, en été et en hiver, alors que les fluctuations de températures du lac sont à leur minimum.

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THIS paper presents some insights into the distribution of fish in areas of thermal discharges by describing the temperatures selected seasonally by four species of fish from western Lake Erie during 2–3 days in a horizontal temperature gradient. Temperature selections of fish naturally acclimatized to ambient environmental conditions each season are reasonable estimates of the reactions of a species to areas of elevated water temperatures. The distribution of fish in an outfall area in Lake Monona during summer corresponded to laboratory determined temperature selections (Neill 1971). In previous studies on temperatures selected seasonally (Fry and Hochachka 1970) fish used in experiments were from laboratory stocks or were acclimated to various temperatures and photoperiods.

Methods

Fish were caught by trapping, shore seining, and hook and line fishing near South Bass Island, Ohio. Although fish were collected in 1–5 m of water, isothermal conditions in the shallow (average 8 m) western basin of Lake Erie (Langlois 1954) resulted in uniform water temperatures at capture sites and laboratory holding facilities on a given date. The lake temperatures used (Fig. 1) were those for Fishery Bay (northwest side of South Bass Island; maximum depth, 4 m) as recorded by the Ohio Division of Wildlife Fish Hatchery. Young fish were primarily underyearlings (UY), although some immature (1+ year old) perch and smallmouth bass were tested during the spring. Fish for winter experiments were caught during November and held about 2 months at ambient lake temperatures until tested. During other seasons most fish were held less than 7 days at lake temperatures. Fish were maintained and tested under indirect natural lighting.

Fish were tested in a horizontal temperature gradient approximately 24 m long and 25 cm deep. The gradient was established in a wooden tank 872 × 79 × 50 cm in which a system of alternating transverse baffles formed a series of 28 compartments. Filtered lake water (1–2 liters/min) passed through 0.6-cm tubing within a cooling reservoir and into the low temperature end of the gradient; the water was then heated progressively by immersion heaters as it flowed to a standpipe at the opposite end of the tank.

A Vicor 500-w heater, ARC static switch relay, and corresponding Jumo thermoregulator maintained a constant water temperature ± 0.15 C in the center of each compartment. Each season a different gradient was established by maintaining the temperature in the center of each compartment 0.5–1.0 C above that of the preceding compartment. During the year, the gradient ranged from near ambient lake temperatures to a maximum of 36 C (Fig. 1). Aeration in each compartment almost eliminated vertical temperature stratification and provided nearly saturated oxygen concentrations, from 6.6 to 11.2 mg O_2/liter.

Prior to testing, each fish or group of fish was held in a tank similar to the gradient apparatus for 24–48 hr at ambient lake temperatures. Test fish were transferred from the observation tank to the compartment of the gradient with the temperature nearest the ambient lake temperature. The number of fish of a given species tested during a season (Fig. 1) ranged from three (smallmouth bass in fall) to 119 (emerald shiners in winter). The aggressive behavior of adult and older underyearling smallmouth bass necessitated testing these fish individually and greatly reduced the number that could be tested during a season. Several underyearling white bass and perch and adult smallmouth bass, which were given access to the entire gradient during fall and winter, explored beyond their critical thermal maximum (Mihursky and Kennedy 1967) and died. Later, the same temperatures were selected safely by others of the same species size group after acclimation in the gradient. After the above mortalities, fish were retained below the suspected upper lethal temperatures, and were allowed into progressively higher temperatures as their modal selected temperatures increased.

Observations of fish distribution were initiated approximately 4–6 hr after fish (1–38 per test) had been introduced into the gradient. The number of fish at each temperature was recorded every 10 min during 80–100 min in the morning, at midday, and just before sunset on each of 2–3 days. A test was defined as a series of observation periods on one group of fish. Tests were conducted on 1–4 groups of young and adult fish of each species each season (Fig. 2). Each group of fish was observed during approximately 2 days (six periods of observations) in summer and fall, 2 or 3 days in spring, and 3 days in winter. In 5-day tests in summer, conducted for three of the species, daily distributions of fish in the gradient were similar (Spearman's rank correlation coefficients). During winter, thermal distributions for the 1st and 2nd days were significantly different, but were similar on 2nd and 3rd days.

Frequency distributions of fish about temperatures in the gradient were graphically analyzed, and nonparametric statistics (Kruskal-Wallis analysis of variance, Spearman's rank correlation coefficients and Kolmogorov-Smirnov analysis from Siegel 1956) were used in further comparisons. For underyearlings and adults of each species, the mode of the distribution for each period of observation was determined and the mode for all observations each season except winter was determined from all the data pooled. With the exception of emerald shiners, the modes reported for winter observations (Fig. 1) were determined from the pooled data for the 2nd and 3rd days. The variation about each temperature selection (mode) was expressed (Fig. 1) by the range for 95% of the pooled observations. Figure 1 contains data

from a number of observation periods during 5-day tests not found in Fig. 2.

Temperatures Selected

The modes of the temperatures selected by adults and underyearlings of the four species during 2–3 days in the gradient (Fig. 1) differed ($P=0.05$) seasonally. Temperatures selected in the four seasons were highest for white (18–30 C) and smallmouth (18–31 C) bass, lowest for emerald shiners (6–23 C), and intermediate for yellow perch (10–29 C). However, the modes for adult yellow perch were lower than those for emerald shiners during fall and spring. Only for smallmouth bass did the modes for winter approach those for summer.

The most frequently occupied temperatures, indicated by the range in 80% of the modal temperatures (C) selected, within the gradient during the various seasons were:

Season	Su	F	W	Sp
White bass				
UY	30–34	28–29	18–21	18–20
AD	30–32	14–25	19–25	16–21
Yellow perch				
UY	28–29	24–31	11–15	17–25
AD	23–26	13–21	12–16	10–14
Emerald shiner				
UY	21–23	13–15	11–13	13–15
AD	22–23	15–18	6–7	16–18
Smallmouth bass				
UY	29–31	26–30	24–28	22–28
AD	30–31	21–27	13–26	18–26

Winter ranges include only modes from selections after the 1st day in the gradient.

The species tested exhibited an average difference of 12 C between temperatures selected in summer

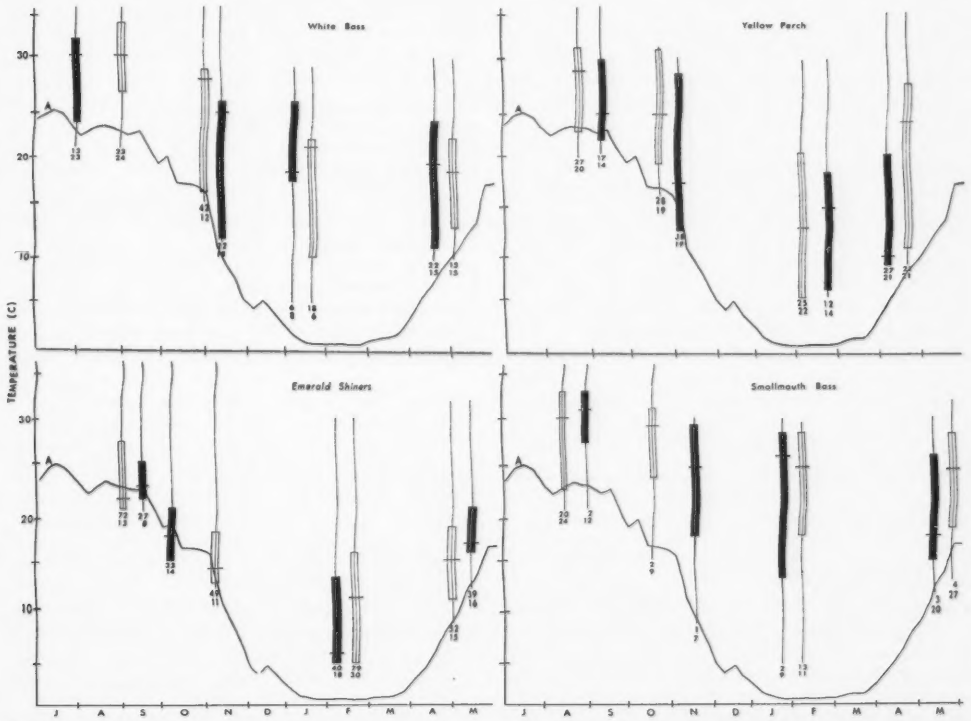

FIG. 1. Temperatures selected each season by adults (solid bars) and underyearlings (open bars) of the four species and (A) weekly averages of ambient lake water temperatures. Horizontal lines, modes from pooled data for thrice-daily observations on 1–4 groups of fish during 2–5 days in gradient; bars, 95% of the observations; vertical lines, ranges of gradient temperatures; numerals below vertical lines, numbers of fish tested (upper) and of periods of observations (lower). Winter data for periods after first day in gradient.

and those selected after the 1st day in winter, with the exception of smallmouth bass. The seasonal shift in selected temperature for all four species did not exceed 17 C. In yellow perch, emerald shiners, and smallmouth bass, adult fish exhibited a greater shift between maximum and minimum temperatures selected than did young fish.

Emerald shiners were the most precise in temperature selections of the four species studied (Fig. 1). The narrowest distributions (95% of the pooled observations) were demonstrated during summer (3 C) by adults and in summer and fall (6 C) by young. The greatest range in temperatures frequented by this species occurred in winter (11 C, UY).

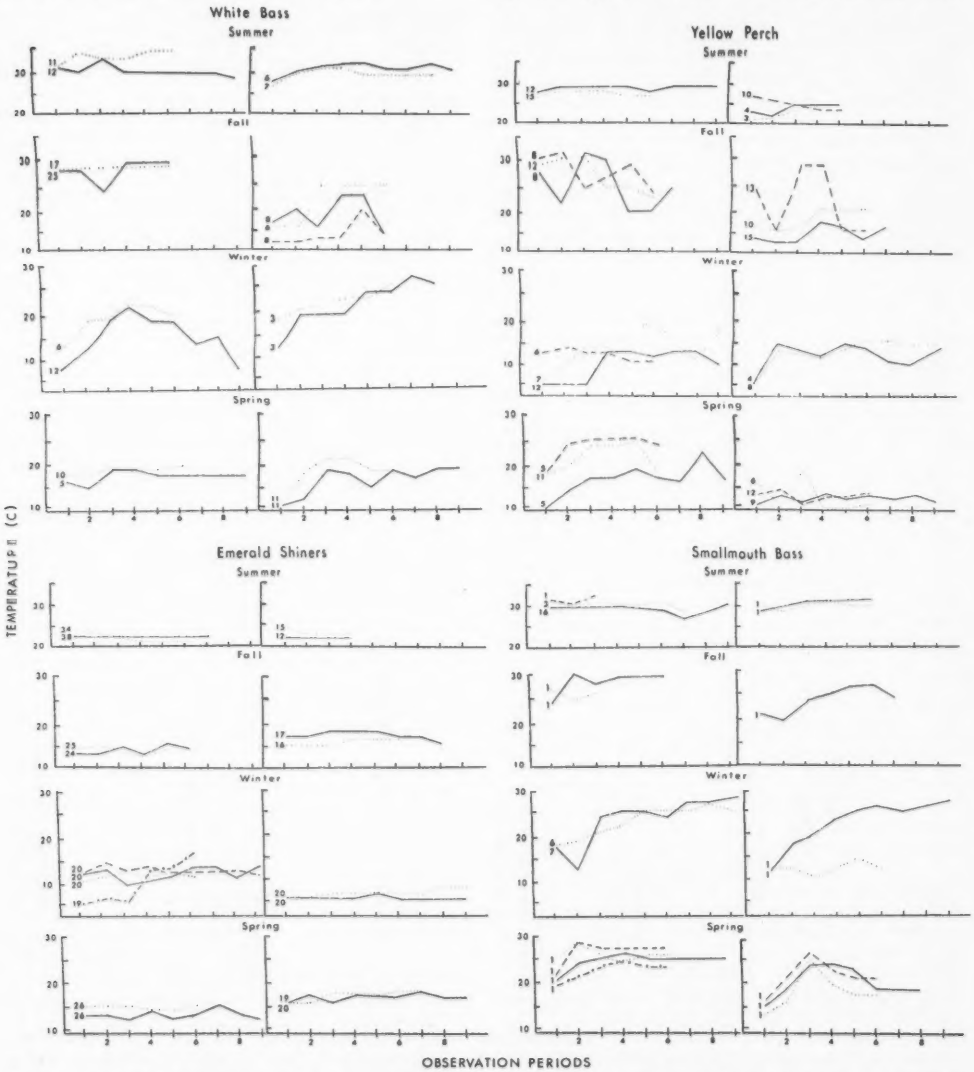

FIG. 2. Modes of temperatures selected each season in successive periods of observations (three per day) by underyearlings (left) and adults (right) of the four species. Each mode is based on 8–10 observations. Numerals to the left of the curves are numbers of fish tested.

Yellow perch were the least precise in thermal preferences. The smallest range in temperatures selected (8 C) occurred in summer (young and adults), and the greatest during winter (15 C, UY) and fall (15 C, AD). Perch behavior was periodically erratic resulting in wide ranges in observed distributions, with fairly representative modal temperature values. Modes of temperatures selected were often close to the upper limits of the observed distributions, especially in underyearling white bass, yellow perch and smallmouth bass.

The modes of temperatures selected by three species changed during 2–3 days in the gradient in fall, winter, and spring, but remained similar over the periods of observations for emerald shiners during all seasons and for other species in summer (Fig. 2). Temperature selections in summer were made in a 1–4-hr period and remained fairly consistent ($P=0.05$) for 2–5 days. Wide fluctuations in modal distributions during fall obscured any trends in preferred temperatures. During the first day in winter tests, white bass, yellow perch, and smallmouth bass selected progressively higher temperatures which differed ($P=0.05$) from those during the subsequent 2 days. Preference temperatures then gradually leveled off below summer selected temperatures, with the exception of underyearling white bass, which selected decreasing temperatures after the 2nd day, and young (13) and adult (1) smallmouth bass, which selected temperatures after 2 days near those in summer. In spring, smallmouth bass, young yellow perch, and adult white bass selected progressively higher temperatures during the 1st day. Other species age groups made relatively stable temperature selections shortly after their introduction into the thermal gradient.

Temperature selections within a given series of observation periods and selections between replicate tests differed least in summer and most in fall or winter (Fig. 2). During all seasons, emerald shiners selected temperatures with a narrow range of modal values. Within a test and between tests, differences between modes ranged from 1–2 C during summer for both young and adults to 5 C (UY) and 3 C (AD) during winter. White and smallmouth bass were intermediate in the ranges of temperatures selected between replicate tests. Differences between modal selected temperatures of yellow perch, the least consistent species, were greatest (16 C, AD) during fall and least (2 C, UY) in summer.

Underyearlings of each species differed ($P=0.05$) from adults in their distributions about modal selected temperatures each season. Differences were most apparent in yellow perch and emerald shiners during summer and winter, when ambient temperatures were fairly constant. Young perch selected temperatures (modes) 4 C or more above temperatures preferred by adults in summer, fall, and spring, but selected 2 C lower in winter. Adult emerald shiners chose higher temperatures than underyearlings in summer, fall, and spring, but about 5 C lower in winter. Clear differences between young and adults were obscured during spring and fall when water temperatures and natural acclimation of fish were changing. The rapid succession of tests (2 weeks or less for each species) during spring reduced differences in thermal history between adults and young. In many cases, tests were closer together in time than could be illustrated in Fig. 1. Differences between the thermal preferences of adults and young were more likely to be related to differences in acclimation during fall, when the period of testing the two age groups of a species ranged to nearly 30 days.

Discussion

The temperatures selected by fish in this study provide some insights into the thermal habitats the species might frequent each season if the lake basin or other waters with similar seasonal ambient temperatures were subjected to thermal discharges. White bass, smallmouth bass, and yellow perch in an environmental gradient of temperatures would probably aggregate during all seasons near the above ambient temperatures selected in the laboratory. Emerald shiners would most likely be attracted to cooler areas of thermal discharges during winter and would completely avoid heated waters in other seasons, especially summer. In a limited gradient with a maximum temperature below the species preference temperature, fish might be found at the highest temperatures available. However, only a small part of a fish population may be affected by small local discharges. Contact with a gradient could occur during seasonal migrations or local movements but may be limited by reduced activity of most species during winter.

The modal temperature selected by underyearling yellow perch during summer in this study (29 C) was well above the mode of 23.4 C (Neill 1971) and maximum of 26.7 C (Ferguson 1958) previously determined in the laboratory, acclimation temperatures being 23–24 C, 20–22 C, and 30 C, respectively. Although yellow perch in Lake Monona avoided an area of warm water discharge above 26.5 C in summer, individuals caught in the heated zone collectively had a median body temperature of about 27 C (Neill 1971). The modal temperature selections made by young perch during spring in the present study (24 C) were similar to the 22–23 C found by Meldrim and Gift (1971) during the same season, and the mode of temperatures selected

during winter by adults was about 6 C less than the final preferendum (21 C) found by McCracken and Starkman (from Ferguson 1958).

The relatively high temperatures selected by underyearling and adult smallmouth bass (30 C) during summer correspond closely to preferred temperatures determined in previous laboratory and field studies. Temperatures selected in laboratory experiments ranged from a final preferendum temperature of about 28 C (Fry 1950 unpublished graphs on temperature preference and cruising speed of largemouth and smallmouth bass, in Ontario Fish. Res. Lab. Library, Toronto) in a vertical gradient to 31 C for dominant smallmouth in a horizontal gradient (Peek 1965). Smallmouth bass in thermal discharges have been caught at maximum temperatures ranging from 33.5 C (Gammon unpublished data 1971, Aquatic survey of the Wabash River, with special reference to the effects of thermal effluents on populations of macroinvertebrates and fish, 1967–1969, DePauw Univ., Greencastle, Indiana) to 36.7 C near a TVA power plant (Churchill and Wojtalik 1969).

A species closely related to the emerald shiner, the spottail shiner (*Notropis hudsonius*), selected a temperature (14 C) in spring (Meldrim and Gift 1971) similar to that selected by emerald shiners (17 C). Although comparison of results from species of the same genus is often acceptable, a mixed species temperature selection test was conducted during summer which demonstrated the wide variation between preferences of species of the genus *Notropis*. Adult emerald shiners and spotfin shiners, *N. spilopterus*, were released at ambient temperatures and within several hours the emerald shiners were grouped about 22 C and the spotfin shiners were at 31 C. The two distributions remained distinct for the 28 hr of the test with no interaction between the two groups. Emerald shiners have been observed in an area of elevated temperatures ranging from 28 to 30 C (Agersborg 1930), which is much above temperatures selected by them during any season in the present study.

Temperatures selected by white bass in this study during summer (30 C) agree with information available on fish distributions in areas of thermal discharges. White bass have been collected at maximum temperatures ranging from 29 C (Hatch 1973) to 34.4 C (Churchill and Wojtalik 1969).

Fish from western Lake Erie appear to select temperatures in a thermal gradient in response to acclimation temperature, which probably has the major effect on thermal preferences (Fry and Hochachka 1970), and a seasonal factor, which may include photoperiod and physiological condition of the fish. Temperatures selected each season after 2–3 days of acclimation to elevated temperatures probably represent final preferendum temperatures, the temperatures ultimately selected in a gradient regardless of previous thermal history (Fry 1947), for that season. While this study describes the effects of combined environmental factors on temperatures selected seasonally by wild fish, previous studies have demonstrated the existence of a seasonal factor under controlled laboratory conditions or with laboratory maintained specimens. Fish which exhibit seasonal temperature selections somewhat independent of acclimation temperature, similar to the four species from western Lake Erie, include brook trout (Sullivan and Fisher 1953), rainbow trout (Mantelman 1958), white perch (Meldrim and Gift 1971), plaice and bitterling (Zahn 1963). The estuarine goby (DeVlaming 1971), barb and guppy (Zahn 1963) do not display seasonal differences in temperature preferences.

Increases in selected temperatures during the first day in the gradient suggest corresponding increases in thermal acclimation, although the rise in acclimation may not have been equivalent to the rise in selected temperature. Modal selected temperatures during winter increased at rates of over 4 C in 24 hr (adult emerald shiners) to nearly 20 C in 24 hr (white and smallmouth bass), while selected temperatures of roach in a similar study increased somewhat slower over a longer period, from 14 C to 23 C in one week (Alabaster and Downing 1960). Observed rates of thermal acclimation of fish in laboratory studies range from 1 to 24 C per day (Fry 1971). Rapid rates of increase in selected temperatures did not occur after the 1st day even during winter, again suggesting that the highest temperatures selected by a species each season were the final preferendum at that physiological condition of the fish. The decrease in modal selected temperatures of underyearling white bass during winter may have represented a change in physiological condition during exposure to the elevated temperatures. Starved fish often select lower temperatures (Dzyan 1959; Javaid and Anderson 1967).

Confinement and limited direction of escape in the experimental apparatus may have contributed to the initial exposure mortalities of underyearling white bass, yellow perch and adult smallmouth bass in our study. A similar inability of young white perch and striped bass to avoid stressful temperatures in a gradient has been termed "low thermal responsiveness" (Meldrim and Gift 1971). Meldrim and Gift also found that the phenomenon caused mortality in only the smallest fish of those tested and occurred most often when ambient water temperatures were changing. Larger fish that explored high temperatures may have had time to respond to the thermal stimulus and escape before

experiencing a change in body temperature. In the present study, low thermal responsiveness was not observed in emerald shiners.

The consistent differences in temperatures selected by young and adults of yellow perch and emerald shiners in this study and of yellow perch in the study by McCauley and Read (1973) and the lack of differences in other species (Alabaster and Downing 1960; Doudoroff 1938; Neill 1971; and Meldrim and Gift 1971) suggest that size or age related differences in temperature preference may vary in importance in defining the thermal habitat of a species. Differences of 4–5 C in thermal preference between juvenile and adult yellow perch (McCauley and Read 1973) were below the 14–8 C differences we found during corresponding spring and fall seasons. Ferguson (1958) suggested that major differences between fish preferences determined in the laboratory and in the field were due to the size of fish considered in each type of investigation. Although his conclusions were based primarily on selections by yellow perch, the information was confounded by seasonal differences.

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Cardiovascular–Respiratory Activity During Recovery from Anesthesia and Surgery in Brook Trout (*Salvelinus fontinalis*) and Carp (*Cyprinus carpio*)

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HOUSTON, A. H. C. L. CZERWINSKI, AND R. J. WOODS. 1973. Cardiovascular-respiratory activity during recovery from anesthesia and surgery in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*). J. Fish. Res. Board Can. 30: 1705–1712.

Brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*) were subjected to handling, anesthesia, and extensive experimental pretreatment, including the emplacement of electrocardiograph electrodes and of buccal, cleithral, dorsal aortic, and urinary bladder catheters. Determination of oxygen consumption, cardiac and ventilatory rates, and rate ratios, ventilatory flow, ventilatory stroke volume, and gross oxygen utilization were subsequently carried out at intervals over a 26-hr postpreparation period. Four general patterns of functional variation were observed during recovery. In the most common pattern, activity changed steadily to a final value. Other patterns were characterized by an initial lag phase or intermediate maximum, minimum, or plateau values prior to apparent stabilization. Brook trout required longer than carp for recovery, and differed from carp in the relationship between ventilation and oxygen utilization during recovery. Provision for recovery periods of not less than 24 hr is recommended for studies involving preexperimental preparations of the type studied.

HOUSTON, A. H., C. L. CZERWINSKI, AND R. J. WOODS. 1973. Cardiovascular-respiratory activity during recovery from anesthesia and surgery in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*). J. Fish. Res. Board Can. 30: 1705–1712.

Nous avons soumis des ombles de fontaine (*Salvelinus fontinalis*) et des carpes (*Cyprinus carpio*) à la manipulation, à l'anestésie et à un traitement préexpérimental intensif, y compris la mise en place d'électrodes ECG et de cathéters dans la bouche, le cleithrum, l'aorte dorsale et la vessie urinaire. Nous avons ensuite mesuré à divers intervalles, sur une période de 26 h après le traitement, la consommation d'oxygène, les taux et les rapports de taux cardiaques et ventilatoires, le débit ventilatoire et l'utilisation brute d'oxygène. Nous avons observé quatre types généraux de variations de ces diverses fonctions pendant le processus de rétablissement. Le type le plus commun est celui dans lequel l'activité change régulièrement pour atteindre une valeur finale. Les autres types de réponses sont caractérisés par une phase initiale de retard ou des maximums, minimums ou plateaux intermédiaires avant qu'apparaisse la stabilisation. Les ombles de fontaine prennent plus de temps à se rétablir que les carpes et diffèrent de celles-ci en termes de relation entre la ventilation et l'utilisation de l'oxygène pendant la période rétablissement. Il est recommandable de prévoir des périodes de rétablissement d'au moins 24 h pour des études impliquant des préparatifs préexpérimentaux du type considéré dans le présent travail.

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HANDLING, anesthetization, and the surgical manipulations now widely used in fisheries work (Klontz and Smith 1968) are known to prompt marked changes in physiological status (Inui 1969; Houston et al. 1969, 1971a,b; Chavin and Young 1970; Garey and Rahn 1970; Hunn and Willford 1970; Wedermeyer 1970). As yet relatively little attention has been given to investigation of time patterns of recovery following such treatments — a question of some importance if valid information is to be obtained through application of these methods. The definition of recovery is complicated by present uncertainties regarding what constitutes physiological normality in fishes. Nevertheless, as a working approximation a specimen may be said to have

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recovered from experimental pretreatment when it has achieved a functionally stable state comparable to that seen in animals not previously subjected to preparative stresses. In situations in which pretreatment values can be obtained it is possible to employ both stability and equivalence criteria. Several of the studies cited fall into this category, and suggest that recovery may require anywhere from a few hours to several days depending upon the specific parameters considered, and the nature and extent of the treatment to which the animals have been subjected.

Because the measurement of most systemic variables requires the prior implantation of electrodes, catheters, telemetry systems, or other devices, estimates of recovery must, in such instances, be based upon a single criterion — attainment of a functionally stable state. With specific reference to cardiovascular–respiratory activities, changes in cardiac and ventilatory rates and dorsal aortic blood pressures have been followed during and for some hours after M.S. 222 anesthesia and aortic catheterization (Randall 1962; Randall et al. 1965). The data obtained indicated that stabilization might require as little as 1–3 hr. The extent and persistence of many of the other sequelae of preparative stress suggested, however, that longer-term studies would be of value. The present investigation, which includes observations upon oxygen consumption, cardiac and ventilatory activity, ventilatory flow, and oxygen utilization in groups of 15 brook trout and carp over a 26-hr posttreatment period was therefore undertaken.

Materials and Methods

Two-year brook trout (mean weight, 518.7±31.0 g) were obtained from a local, commercial hatchery, and carp (mean weight, 591.8 ± 28.1 g) from nearby drainages through the cooperation of the Wisconsin State Department of Natural Resources. All stocks were housed in continuous-flow, self-flushing 250-gal fiberglass tanks, and fed twice daily on a pelletted trout ration (Glencoe Mills). All stocks were initially held at 5–7 C and exposed to a 12–12 light–dark photoperiod. Brook trout were acclimated to 10.4 ± 0.3 and carp to 14.8 ± 0.4 C for not less than 3 weeks before use, and all tests were carried out at acclimation temperatures.

Preparation of Specimens

Specimens were subjected to preparative stresses similar to those used in earlier studies in this series (Houston et al. 1969, 1971a, b, 1972). Following anesthetization in freshly-prepared, aereated tricaine methane sulphonate (Ayerst "Finquel®," 100 mg/liter) for a standard 15-min period at acclimation temperature specimens were transferred to an operating assembly. Irrigation with fresh anesthetic of the same concentration was carried out at flows of approximately 1 liter/min. Each was then fitted with three electrocardiograph pin electrodes and functional cleithral, buccal, and urinary bladder catheters. Dorsal aortic catheters were also emplaced. No attempt was made to ensure that the latter were operational, as the primary objective was only to impose levels of stress similar to those to which other specimens in these studies had been subjected. Accordingly, all aortic catheters were cut short and plugged. Preparation time varied from 17 ± 1 min in trout to 23 ± 2 min for carp. The animals were then transferred in water to U-shaped plastic holding cradles (Fig. 1) and loosely restrained in these by elastic bands.

Determination of Oxygen Consumption and Cardiovascular–Respiratory Activity

The respirometer (Fig. 1) used was a modification of the constant pressure head design developed by Beamish and Mookerjii (1964) and incorporated specimen restraint features similar to those described by Holmes and Stainer (1966) for a renal function test chamber.

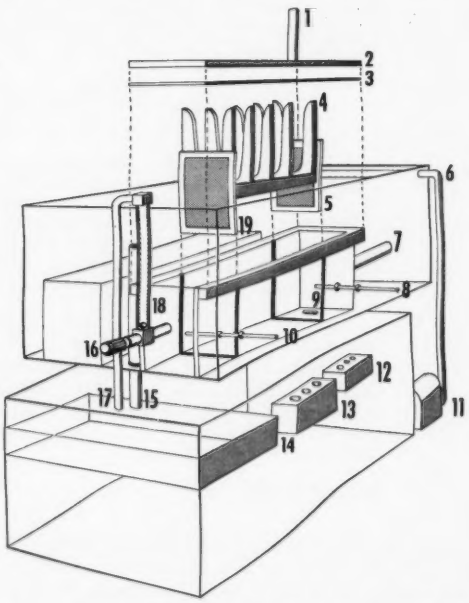


FIG. 1. Respirometer. 1, chamber top venting tube; 2, chamber top; 3, gasket; 4, restraining cradle; 5, anterior partition screen; 6, water input; 7, connection to gas-stripping column; 8, input sampling port; 9, magnetic stirrer; 10, output sampling port; 11, pump; 12, holding bracket for thermister probe and oxygen and *p*H electrodes; 13, holding bracket for water sampling bottles; 14, filter bed; 15, upper tank standpipe; 16, flowmeter regulating valve; 17, overflow from flowmeter; 18, flowmeter; 19, posterior partition screen.

Flow through each chamber was controlled by means of a valved Gilmont flowmeter with a rated accuracy of ±2%. Oxygen consumption was estimated by determination of the oxygen content of water samples drawn from input and output sampling ports at known flow rates using the phenyl–arsene oxide procedure. To minimize specimen disturbance during recording the system was housed in a walk-in cold room and the upper chambers masked with neutral-toned opaque paper. As transducer cables were ported through the wall of this room to the polygraph it was necessary to enter the room only during collection of water samples and determination of flow rates. As judged from records of cardiac and ventilatory activity specimens did not appear to be excessively disturbed by this.

Following preparation each specimen in its restraining cradle was fitted into a respirometer chamber. Catheters and electrode cables were vented through the chamber top, and the latter sealed to its hull by siliconized welding clamps. Statham P23BC pressure transducers, linked through Grass 7P1 preamplifiers to a Grass Model 7 polygraph, were used to record buccal pressure oscillations. Electrocardiograms were recorded on the same unit via TP4 tachograph preamplifiers.

All experiments were initiated between 8 and 9 AM, and continued for 26 hr. Except as noted, 15 fish of each species were used at each test interval. Records of ECG patterns and buccal pressure variations were begun as quickly as possible and continued for the immediate 0.5-hr postpreparative period; in some instances up to about 20 min, fewer than 15 sets of recordings were obtained. Thereafter records were made for 10-min intervals bracketing 1-, 1.5-, 2-, 3-, 6-, 12-, 24-, 25-, and 26-hr recovery times. Determinations of water temperature, chamber flow rates and input, output and postbranchial oxygen levels were carried out after each recording period. Ventilatory flow was estimated by the Fick procedure, and gross oxygen utilization as described by Randall (1970).

The estimates of ventilatory flow, stroke volume, and utilization are primarily of relative value. All such calculations assume that water samples drawn from postbranchial catheters are representative of a mixed expiratory flow. Davis and Watters (1970) have challenged the validity of this assumption with reference to the siting of catheters on the opercular of trout. Their results clearly demonstrate that values for expiratory oxygen tension are highly dependent upon sampling position. Thus, although subsequent studies by Dr. J. C. Davis (personal communication) suggested that cleithral catheter sites produced less variable results than did those on the operculum, calculations based upon expiratory oxygen data should be viewed with caution. However, the values for ventilatory flow and utilization obtained in the present study compared favorably with those reported by Davis and Cameron (1970) for rainbow trout following the use of a system providing for a catheterless means of evaluating ventilatory flow.

As no differences significant at $P<0.05$ were found among the data collected at 24–26 hr, these values were pooled.

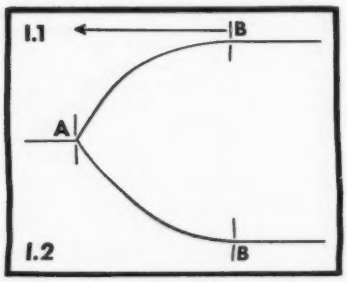
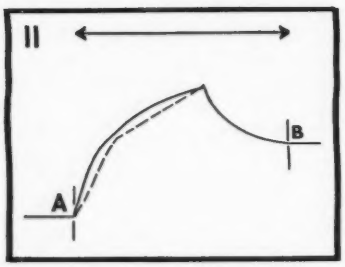
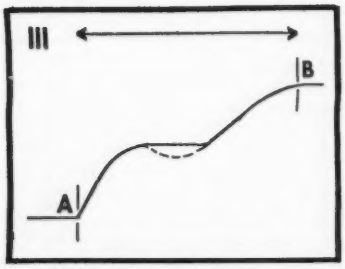
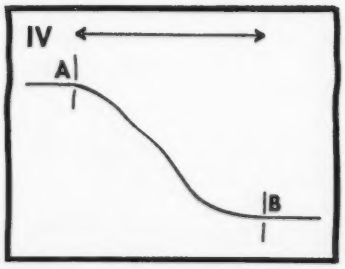
Variation in Systemic Activity

While the systemic manifestations of the recovery process were complex in character, most of the variations observed can be grouped into four general categories with respect to the overall trends which they exhibit (Table 1).

The most commonly encountered pattern (category I) was one in which variation with time followed a relatively steady course to a final value. Four of the seven parameters followed in carp, and two in trout, at least approximate a situation of this type. With one exception, — cardiac rate in carp — apparent stabilization times ranged from 2–3 hr. The second most prevalent pattern may represent, as do categories III and IV, a variant on the first and is characterized by an intermediate peak before decline to stable levels. Recovery patterns of this kind were encountered only in brook trout, and were associated with stabilization times of much greater duration than were typical of the category I pattern, i.e. 12–24 hr. The third general form of response was distinguished by an intermediate period during which activity varied little or actually declined before rising to a final value. Such was the case with gross oxygen utilization and ventilatory stroke volume in carp, and cardiac rate in trout. Again, estimated stabilization times exceeded those of the category I pattern, ranging from 6 to 24 hr. Finally, the fourth type of recovery pattern appeared to be a variant on category I.2 in the sense that it involved a lag phase of significant duration before decline to a final value. This form of response was seen only with respect to ventilation rate in carp and involved a stabilization period of from 12 to 24 hr.

Of the several points which emerge from this pragmatic characterization of temporal changes in systemic activity during recovery from experimental pretreatment, one is of obvious importance. While several functions (oxygen consumption, cardiac rate, ventilation-to-cardiac rate ratio and ventilatory flow in carp; ventilation rate and ventilatory stroke volume in trout) seemingly stabilize within 2–3 hr, a number of others require up to 24 hr. Moreover, responses falling into categories III and IV were associated with periods, sometimes of several hours duration, during which activity varied little. Cardiac rate in brook trout and ventilation rate in carp represent striking examples of this, since the periods of "deceptive stability" were as much as 6–12 hr in duration. On this basis it seems clear that the design of studies involving extensive surgical manipulation of experimental animals should include provision for recovery periods of much longer duration than is commonly the practice. Against the desirability of this must be balanced the practical difficulty of, for example, maintaining

TABLE 1. Categories of variation in systemic functions, in decreasing order of frequency, during recovery of trout and carp from anesthesia and experimental preparation.

	Category description	Parameters approximating category	Estimated stabilization time (A-B) (*hr*)
	A smooth curve ascending (1) or descending (2) to a final value.	1. Carp: oxygen consumption	1–1.5
		ventilation-to-cardiac rate ratio	2–3
		ventilatory flow	1–1.5
		Trout: ventilation rate	1–1.5
		ventilatory stroke volume	1.5–2
		2. Carp: cardiac rate	3–6
	A possible variant on I. A curve ascending smoothly or in two phases to a single maximum and declining to a final value.	Carp: none	
		Trout: oxygen consumption	12–24
		ventilation-to-cardiac rate ratio	12–24
		ventilatory flow	12–24
		oxygen utilization	12–24
	A possible variant on I (1). A curve ascending to a final value with an intermediate minimum or plateau.	Carp: ventilatory stroke volume	6–12
		oxygen utilization	12–24
		Trout: cardiac rate (?)	12–24
	A possible variant on I (2). A curve descending to a final value following an initial lag phase.	Carp: ventilation rate	12–24
		Trout: none	

vascular catheters in position and patent, as well as problems of tissue lesioning and necrosis in long-term preparations (Kirsch 1972). Nevertheless, the observations of the present study, taken in conjunction with earlier findings upon posttreatment variations in hematological status and water–electrolyte balance (Houston et al. 1969, 1971b), suggest the need for recovery periods of not less than 24 hr and preferably longer.

Comparison of Carp and Brook Trout

The data also indicate that carp are either more tolerant of preparative stresses or have the capacity to recover more rapidly from imposed stress than do brook trout. In carp, oxygen consumption, ventilatory flow and ventilation-to-cardiac rate ratios achieved reasonably stable levels within 2–3 hr. Two other parameters (cardiac rate, ventilatory stroke volume) had apparently stabilized by 12 hr, while only two (ventilation rate, gross oxygen utilization) required up to 24 hr. By contrast, in trout only two of the seven parameters (ventilation rate, ventilatory stroke volume) followed the category I pattern. The remaining five fell into either category II or III, with stabilization times of 12–24 hr.

Preliminary analysis suggested that the trout also differed from carp in terms of the relationship

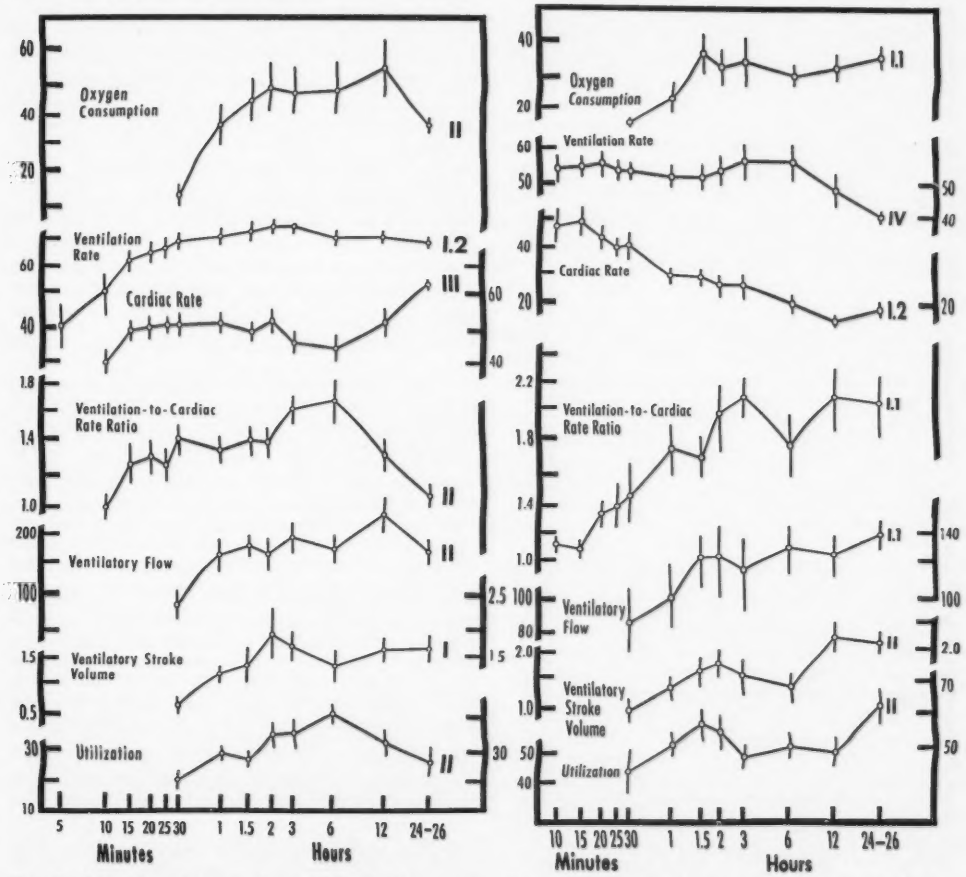


FIG. 2. Variations in systemic activities during recovery from handling, anesthesia, and surgery in brook trout (left) and carp (right). With minor exceptions values at each test interval are for 15 fish. Oxygen consumption in mg kg^{-1} hr^{-1}; ventilation rate, cycles/min; cardiac rate, contractions/min; ventilatory flow, ml kg^{-1} min^{-1}; ventilatory stroke volume, ml; and gross oxygen utilization, %. Circles and vertical lines represent means ± one standard deviation. Roman numerals refer to response pattern categories (Table 1).

between oxygen consumption and utilization and ventilatory flow during recovery. To examine this in more detail, data were grouped in terms of an immediate recovery period (0.5–1.5 hr) during which oxygen consumption increased rapidly, an intermediate phase (2–6 hr) when consumption was relatively stable, and the final 24–26-hr period. Studies reported by Randall and his co-workers (Stevens and Randall 1967; Holeton and Randall 1967) suggest that relationships between these variables can be approximated by linear functions over the relatively low ventilatory flow ranges observed in this study. Accordingly, linear regression equations and correlation coefficients (Table 2) were calculated.

The expected direct relationship between flow and oxygen consumption was found in carp during the three designated periods. The equations for the 2–6 and 24–26-hr recovery periods were not statistically distinct. Both were, however, characterized by higher y-intercept and lower slope values than was the case for the 0.5–1.5-hr interval. The relationships between flow and utilization were also essentially similar during all periods of recovery, having the anticipated negative form and being characterized by similar slope (–0.14 to –0.16) and intercept (68.8–74.4) values. Among trout, changes in the flow-to-consumption relationship followed a somewhat similar pattern in the sense that intercept increased while slope declined as recovery progressed. However, the change in utilization vs. flow in brook trout was distinctly at variance with the situation observed in carp. During the 0.5–1.5-hr period utilization was less than 30% and seemingly independent of ventilation. From 2 through 6 hr utilization rose to about 40%, but bore no significant relation to flow. Only after 24 hr was the utilization-to-flow relationship comparable to that found throughout the recovery process in carp. Absence of an inverse relationship between oxygen utilization and branchial ventilation has been previously reported. Stevens and Randall (1967) for example, observed this in trout exercised at 5 C, and it has also been encountered in a variety of other teleosts under circumstances in which ventilatory flow rates have been increased in response to forced exercise or hypoxia (Shelton 1970). It is uncertain as yet which elements in the total exchanger complex contribute to this phenomenon, and no explanation for its occurrence during the recovery of trout can be offered.

TABLE 2. Relationship of oxygen consumption ($\dot{V}_{O_2}$, mg kg^{-1} hr^{-1}) and utilization (U,%) to ventilation ($\dot{V}_I$, ml kg^{-1} min^{-1}) during recovery of brook trout and carp from anesthesia and experimental preparation.

Time period (*hr*)	Regression equation	Correlation coefficient
	Carp	
0.5–1.5	$\dot{V}_{O_2}(\pm 3.42)=-1.2+0.29\dot{V}_I$	0.93[a]
2–12	$\dot{V}_{O_2}(\pm 2.65)=10.3+0.20\dot{V}_I$	0.58[a]
24–26	$\dot{V}_{O_2}(\pm 3.00)=11.2+0.20\dot{V}_I$	0.71[a]
0.5–1.5	$U(\pm 3.08)=74.4-0.16\dot{V}_I$	–0.58[a]
2–12	$U(\pm 2.34)=68.8-0.14\dot{V}_I$	–0.48[a]
24–26	$U(\pm 3.77)=73.1-0.15\dot{V}_I$	–0.42[a]
	Brook Trout	
0.5–1.5	$\dot{V}_{O_2}(\pm 2.68)=3.6+0.13V_I$	0.69[a]
2–12	$\dot{V}_{O_2}(+4.49)=-3.8+0.25V_I$	0.67[a]
24–26	$\dot{V}_{O_2}(\pm 1.68)=18.4+0.06V_I$	0.39
0.5–1.5	$U(\pm 2.08)=27.5-0.00V_I$	–0.01
2–12	$U(\pm 2.28)=39.8-0.02V_I$	–0.12
24–26	$U(\pm 3.09)=55.0-0.14V_I$	–0.51[b]

[a]$P < 0.01$.
[b]$P < 0.05$.

While any discussion of the basis of variations in cardiovascular–respiratory activity during recovery must be regarded as speculative at the present time, a number of recent studies give some insight as to the possible mechanisms involved. Houston et al. (1971a) have demonstrated that several features of cardiovascular–respiratory function are significantly altered within 1–3 min of exposure to anesthetic. The time course of observed change suggested that Finquel® exerts an immediate depressive influence upon central autonomic areas, followed by subsequent peripheral effects. Transfer of specimens to anesthetic-free water leads to rapid clearance of anesthetic from the blood (Houston and Woods 1972), the time course of clearance being comparable to the stabilization times associated with category I parameters. Significant anesthetic residues remain, however, for several hours (Walker and Schoettger 1967), and this is particularly true of brain tissue (Hunn personal communication 1971). Thus, it is not unlikely that the anesthetic may also exert a persistent action upon central regulatory activities corresponding to the longer-duration changes seen in categories II, III, and IV.

From another viewpoint recent reviews (e.g. Shelton 1970) suggest that ventilation and cardiac activity in fishes are largely regulated by reference to vascular levels of oxygen and carbon dioxide and presumably body fluid *p*H, when ambient tensions are normal. While no information is as

yet available upon the variation of either gas during recovery one would anticipate a condition of hypoxia coupled with hypercapnia during sustained anesthesia. With respect to acid-base balance Garey and Rahn (1970) have reported that carbon dioxide tension, bicarbonate and *p*H are significantly altered following anesthesia and surgery upon the air-breathing electric eel. Periods of some 10–12 hr were required for return of these to stable values, intervals comparable to those seen with respect to at least some of the stabilization times encountered in the present study.

Finally, a number of studies point to the likelihood of a substantial endocrine involvement during the recovery process. Leloup-Hatey (1958), Chavin and Kovacevic (1961), Nakano and Tomlinson (1967), Fagerlund (1970), and Chavin 1970 (personal communication) have shown that a variety of stress situations prompt the rapid release of corticosteroids and catecholamines, and that these can be correlated with persistent effects upon several physiological and biochemical activities. That the former class of hormones, at least, influence cardiovascular activity has been well established (Randall 1970). Accordingly, the possibility of a stress-induced endocrinological involvement in the recovery process cannot be disregarded.

Acknowledgments

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Myxosoma cerebralis: Isolation and Concentration from Fish Skeletal Elements — Trypsin Digestion Method

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LANDOLT, M. L. 1973. *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements — trypsin digestion method. J. Fish. Res. Board Can. 30: 1713–1716.

An improved method for detection of *Myxosoma cerebralis* spores is described. Spores were freed from skeletal tissue by trypsin digestion and ether was used for purification of spore suspension. As compared to mechanical grinding, this procedure resulted in a sixfold increase in observable spores.

LANDOLT, M. L. 1973. *Myxosoma cerebralis:* isolation and concentration from fish skeletal elements — trypsin digestion method. J. Fish. Res. Board Can. 30: 1713–1716.

Nous décrivons une méthode améliorée pour la détection des spores de *Myxostoma cerebralis*. Les spores sont dégagées du tissu squelettique par digestion tryptique, et on utilise l'éther pour purifier la suspension de spores. Comparée au broyage mécanique, cette méthode produit une augmentation de six fois du nombre de spores observables.

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WHIRLING disease, caused by the histozoic parasite *Myxosoma cerebralis* (Protozoa: Myxosporidea), is a disfiguring and often fatal disease of salmonid fishes (Hoffman et al. 1962). The trophozoites localize in the cartilage of young fish and undergo sporulation (Hoffman et al. 1962). The mature spores then become trapped as the skeleton ossifies, to be released primarily when the fish dies and decays, and secondarily to be shed with fecal products (Hoffman et al. 1962).

While heavy infections of whirling disease are easily diagnosed by identifying the spores in minced skeletal tissue (Hoffman 1970; Lom and Hoffman 1971; MacLean 1971), light infections or carrier states with low numbers of spores are difficult to detect with present methods. For this reason, a method for obtaining pure concentrations of spores has been sought that would facilitate the visible detection of the parasite.

The most important defense mechanisms against invasion by pathogens in fishes are nonspecific factors such as the body covering and the *p*H of intestinal fluid (Lom 1969). Fish do, however, also possess specific internal inflammatory responses (Finn and Nielson 1971), and immunoglobulins (Summerfelt 1966; Snieszko 1970), and immunization of fish against certain bacteria has been well documented (Klontz and Anderson 1970). An immune response to protozoa is well known in the higher vertebrates (Garnham et al. 1963), and evidence has been presented for a host response by white catfish (*Ictalurus catus*) to the ciliate *Ichthyophthirius multifiliis* (Bauer 1959; Beckert and Allison 1964). No immunological work has been done with myxosporideans; however, Coccidia evoke a specific immune response in domestic animals (Horton-Smith et al. 1963). Salmonids may well produce antibodies on exposure to *M. cerebralis*. A pure spore suspension will be required for the serological detection of exposed fish.

Since spores of this parasite are embedded singly throughout the bone and cartilage, and seldom in discrete granulomas, the preparation of pure spore suspensions is difficult.

MacLean (1971) outlined a procedure for detecting spores. Prasher et al. (1971) described a second procedure which yielded concentrations of up to 70,417 spores/ml. Neither procedure, however, gave pure suspensions of spores. Preliminary work at this laboratory indicated that concentration by sedimentation, density gradient, filtration, or sonication is not satisfactory for obtaining pure preparations of spores. The need for chemical digestion of the skeletal elements was apparent; two enzymes, pepsin and trypsin, proved to be the most effective for releasing spores from cartilage. Rydlo (1971) described the use of pepsin but expressed his results qualitatively rather than quantitatively. Markiw

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TABLE 1. Increase in total number and concentration of *Myxosoma cerebralis* spores at each step of digestion procedure, based on the mean values obtained from 20 fish.

Treatment	Total no. spores per lot	Increase over initial count	Concn spores/ml	Increase over initial concn
Decalcification (initial count)	325,000	–	9,365	–
Trypsin, 12 hr	612,000	1.88 fold	61,200	6.53 fold
Trypsin, 24 hr	1,525,000	4.69 fold	152,500	16.28 fold
Acidification	1,750,000	5.38 fold	175,000	18.69 fold
Etherization	1,340,000	4.12 fold	134,000	14.31 fold
Final washing	1,840,000	5.66 fold	184,000	19.68 fold
Pelletting	1,840,000	5.66 fold	101,200,000	10,800.00 fold

(personal communication) described the use of pepsin followed by trypsin, with well quantified results.

The purpose of this paper is to describe the use of trypsin to implement release and allow concentration and purification of spores. This technique results in a new method of detecting spores, and it provides a source of antigen which will permit the exploration of immunological–serological procedures for a more rapid and sensitive detection of carrier or subclinically infected fish.

Methods

Rainbow trout (*Salmo gairdneri*) ranging in size up to 15 cm in fork length were killed and immediately placed in 45 C water for 10 min to soften the flesh (MacLean 1971). The skeletal elements, with the exception of the fin rays, were then removed, weighed and placed in Decal®, a commercial decalcifying agent. Since spores of *M. cerebralis* did not survive at a *p*H lower than 2.0 it was necessary to raise the *p*H of Decal® to 2.0–2.5 with 0.1 N NaOH. For purely cartilaginous tissue 30 min was sufficient, but with increasingly bony tissue the time required to decalcify the tissue increased to as much as 12 hr. The softened tissue, after being washed in distilled water, was ground in a mortar and pestle or pushed through a garlic press in order to expose maximal surface area to the digestion fluid. The ground tissue was diluted 1:10 with water and duplicate spore counts made with a hemacytometer.

The digestion fluid consisted of 2.0% (w/v) solution of trypsin in Hanks' Balanced Salt Solution. The *p*H of the solution was adjusted to 8.0–8.5 with 0.1 N NaOH and buffered with Tris® at *p*H 8.0. It was found that spore survival was very low at *p*H 9.0 or higher. To avoid bacterial growth, potassium buffered penicillin G (10,000 IU/ml) and streptomycin sulfate (10,000 μg/ml) were added to the digestion fluid. Fresh trypsin was prepared for each day's use and clarified by pressure filtration through a Millipore microfiber prefilter.

The minced, decalcified tissue was placed in the trypsin in a ratio of one part tissue to 100 parts digestion fluid, and stirred constantly at 37 C for 18–24 hr. After digestion, the solution was cloudy and contained considerable tissue debris.

The material was centrifuged at 800 g for 10 min at 25 C and the excess fluid decanted leaving 10–20 ml for easy handling. Using 0.1 N HCl, the *p*H of the solution was gradually lowered to 3.0. As the *p*H approached 4.0 the dissolved proteins were precipitated in a flocculent form. If the drop in *p*H were too sudden the internal anatomy of the spores was converted to a homogeneous mass. To this turbid solution an equal volume of ethyl ether was added. The ether: spore suspension ratio could not exceed 1:1 because at low *p*H ether is more soluble and the spores became homogeneous internally. The ether was thoroughly mixed with the solution and allowed to stand. As the ether rose to the top, all of the proteinaceous precipitate, whose buoyant density may have been altered, was carried up with it, leaving a clear, pure spore suspension in the bottom of the container. This clear suspension was immediately withdrawn, centrifuged at 800 *g* for 10 min at 25 C and the solution was decanted. The spores were resuspended in distilled water and centrifuged (800 *g*, 10 min 25 C) twice, after which the spores were resuspended in Hanks' BSS and stored at 4 or –20 C.

Davis 1925 and Hoffman et al. 1962 speculated that the number of spores formed in any given fish is fixed and that some spores are shed as the fish ages. Young fish, therefore, were considered preferable to mature fish both for their greater ease in decalcification and higher ratio of spores per gram of tissue. Only fish that were 1-year-old or younger were used in this series of tests.

This process takes 18–24 hr for its completion; however, only a few hours of the biologist's time are actually required. This method also diminishes the amount of time that has previously been spent in the tedious process of microscopically looking for spores.

Results

To compare the efficiency of recovery of spores using mechanical means and using trypsin digestion, two lots of 10 fish were employed. All of these fish were the same age, had been exposed to *M. cerebralis*

simultaneously, and maintained in the same aquarium. In the first trial, each of the 10 fish was processed separately and duplicate spore counts were made after each step of the procedure. The initial count represented the number of detectable spores following mechanical disruption of the tissue. In the second trial, the skeletal tissues from all 10 fish were pooled and then divided into 10 subsamples of equal weight in an attempt to minimize differing degrees of infection among the fish. Again, duplicate spore counts were made after each step of the digestion procedure. The combined mean values from these two trials are presented in Table 1. After tryptic digestion and ether separation, approximately a sixfold increase over the initial total number of recoverable spores was achieved. Centrifugation of the spore suspension yielded a packed cell volume of 1,840,000 spores in 0.18 ml. Since the initial concentration was 9,365 spores per ml, the final concentration of 101,200,000 spores per ml represented about an 11,000-fold increase in concentration (spores per milliliter).

Since it is not possible to naturally infect fish with a known number of spores, 100,000 spores were added to each of 10 uninfected skeletons prior to mincing of the tissue. Obviously, this was an artificial situation and could not be compared with infected material; however, this was intended only as a control measure to help estimate spore loss during the procedure. At the termination of the digestion procedure, a mean number of 89,600 spores were recovered from each fish and represented approximately a 90% recovery. This indicated that with careful handling and a minimal number of transfers there should theoretically be little loss of available spores.

At the termination of the purification process the spores were judged to be alive and undamaged in that the sporoplasm, polar capsules, and polar filaments were intact. At this time the mucopolysaccharide envelope could also be demonstrated after staining with India ink. The same conditions prevailed after repeated freezing and thawing of the spore suspension.

Discussion

The described procedure provides a new method for the detection of *M. cerebralis* infections. This process has two potential uses. First of all, it is especially valuable in cases in which there are low numbers of spores that might escape detection by purely mechanical means. By increasing the total number of recoverable spores by a factor of six, the hatchery biologist who is called upon to certify certain lots of fish to be free of whirling disease consequently increases his chances of recognizing low-grade infections. Secondly, a potential source of antigen has been made available. Because the mucus envelope is intact, because the spore wall is not visibly disrupted, and because the internal anatomy is not visibly altered after digestion and purification, hopefully, the antigenic determinants on the surface of the spore as well as those contained within the spore are intact and serological detection of whirling disease will soon be implemented.

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Analysis of Energy Flow and Production in an Unexploited Marine Flatfish Population[1]

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MacKinnon, J. C. 1973. Analysis of energy flow and production in an unexploited marine flatfish population. J. Fish. Res. Board Can. 30: 1717–1728.

The seasonal pattern of production processes in an unexploited resident population of American plaice (*Hippoglossoides platessoides*) in St. Margaret's Bay, N.S., was analyzed with an energetics model which represents an extension of the analytical approach used in fishery theory. During summer, production is about twice the annual net production of 1.5 kcal/m² by fish aged 1 and up. The ecological efficiency is 17%, with larvae and 0+ fish accounting for some 20% of total population ingestion and 34% of net population production. Metabolic expenditures constitute the largest fraction (62%) of population energy intake and about 80% of this amount is consumed during summer. Plaice ingest about half the yearly estimated production (25 kcal/m²) of benthos in the deeper parts of the Bay.

MacKinnon, J. C. 1973. Analysis of energy flow and production in an unexploited marine flatfish population. J. Fish. Res. Board Can. 30: 1717–1728.

L'auteur analyse le schéma saisonnier des processus de production chez une population résidente inexploitée de plies canadiennes (*Hippoglossoides platessoides*) de la baie St. Margaret, Nouvelle-Ecosse. Il utilise à cette fin un modèle d'énergétique qui est un prolongement de la méthode employée dans la théroie des pêches. En été, la production est environ deux fois plus grande que la production nette de 1.5 kcal/m² réalisée par les poissons de 1 an et plus. L'efficacité écologique est de 17%, les larves et les poissons âgés de 0+ étant responsables de quelque 20% de l'ingestion totale par la population et de 34% de la production nette de la population. Les dépenses métaboliques sont responsables de la majeure fraction (62%) de l'absorption d'énergie par la population, et environ 80% de cette quantité est consommé en été. La plie canadienne absorbe environ la moitié de la production annuelle estimée (25 kcal/m²) de benthos dans les secteurs profonds de la baie.

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THE concept of a fish population as a self-regulating system, exchanging energy and materials with its environment and tending towards a steady state, is fundamental to the theory of fisheries (Nikolsky 1969). Most theoretical effort in this field (e.g. Beverton and Holt 1957) has concentrated, however, on analysis of the relationships between one component of energy flow, the fishery yield, and the various parameters describing the exploited phase of the population. In extending the theory to include more ecological aspects (Paloheimo and Dickie 1970; Gulland 1971) it is important to evaluate other energy flows such as those associated with the processes of metabolism and food consumption, and to consider the whole population, including its unexploited phase. Given this type of information it is possible to test, for example, the hypothesis of Nikolsky (1969) that exploited fish populations actively respond to disturbances by means of mechanisms which tend to balance the population's food demand and its supply.

Estimates are available for the complete energy flow pattern in a number of freshwater fish populations, following the early work by Mann (1965), but there is almost no information of this type for marine fish. The few marine studies include Lasker's (1970) analysis of a Pacific sardine (*Sardinops caerulea*) and the investigation by Steele and Ed-

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wards (1970) on O-group plaice (*Pleuronectes platessa*) and dabs (*Limanda limanda*) in Loch Ewe.

This paper describes an analytical production model and the seasonal energy storage and flow patterns that it predicts for an American plaice (*Hippoglossoides platessoides*) population in St. Margaret's Bay, N.S. The population is unexploited and, except for movement of eggs and larvae during exchange of surface water with offshore, there is apparently no net migration (Bakken 1973). Since this represents a rare situation for a commercially important species, the pattern of energy flow in this population is of particular interest.

Analytical Model

Given an initial steady state, the model generates the seasonal patterns of energy storage and flow for individual fish, for each age-class, and for the population as a whole. This type of analysis is suited to populations of plaice and other species in which individual fish undergo an annual cycle involving significant variations in growth rate, metabolism, and food consumption. An important feature of the model is the use of a metabolic submodel instead of a constant multiplier as suggested by Winberg(1956) for estimation of the metabolism and hence food requirements of fish under field conditions. The population is considered to have a stable age distribution and to consist of a locally resident stock with no net immigration.

Model Structure

Processes in an individual fish — The metabolic submodel is derived from the energy conservation equation (Winberg 1956) applied to an individual fish.

Metabolism is considered to consist of several distinct components (Kerr 1971) with values which depend upon the rates of growth and food intake (Fig. 1). These components include the energy requirement for standard metabolism (Fry 1966), the energy requirement for activity and the energy released as heat during food processing (i.e. specific dynamic action). Additional assumptions are: (1) standard metabolism depends upon fish size, season, and temperature but is independent of feeding level; (2) activity metabolism for foraging and swimming is proportional to standard metabolism at starvation and increases linearly with total metabolic level; (3) food is processed with a constant conversion efficiency (see equation 2) which is similar for standard and activity metabolism and for somatic and gonad energy storage requirements; (4) during starvation, energy may be transferred from body energy stores and converted to gonad materials with a certain constant efficiency.

Standard metabolism, M_S, is computed from an expression relating laboratory measurements of the rate of oxygen consumption and weight at different temperatures and seasons. In accord with the assumption mentioned above, activity metabolism is given by

$$M_A = a_1 M_S + a_2(M - M_S) \quad (1)$$

where a_1 and a_2 are constants and M is total metabolism.

For each energy requirement E, an amount of food energy (E/ϵ_p) must be processed and a quantity of energy $((1-\epsilon_p)/\epsilon_p)E$ released as heat. This follows if one defines food processing efficiency, ϵ_p, as the ratio of an energy requirement, E, to the energy of food that must be processed to meet this requirement. The amount of heat released during food processing, Q_p, to meet all requirements for an individual fifh is

$$Q_p = ((1-\epsilon_p)/\epsilon_p)(M_S + M_A + G_B + G_G) \quad (2)$$

where G_B and G_G are the amounts of energy storage in body and gonad components, respectively.

When the feeding level is above maintenance, the total metabolic energy expenditure for a particular time interval is determined from

$$M = M_S + M_A + Q_p \quad (3a)$$

or, when equations 1 and 2 are introduced, from

$$M = (b_1 M_S + b_2(b_1 M_S + G_B + G_G))/b_3 \quad (3b)$$

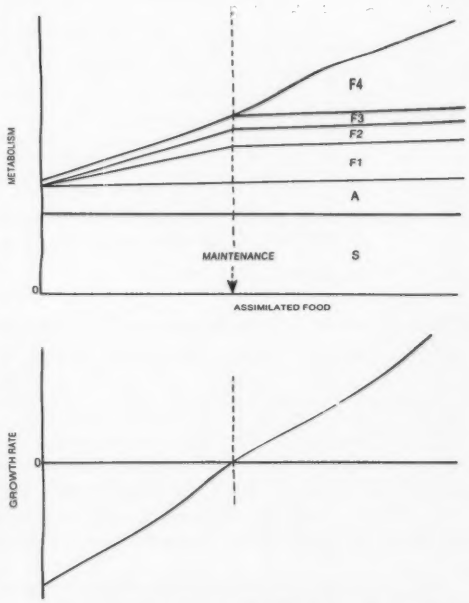

FIG. 1. The metabolic submodel: relationships between daily metabolism, growth, and assimilated food. S=standard metabolism; A=activity metabolism; F=food processing heat for standard (F1), activity (F2), gonad growth (F3), and body growth (F4) requirements.

where $b_1 = 1 + a_1 - a_2$; $b_2 = (1-\epsilon_p)/\epsilon_p$; and $b_3 = 1-a_2-a_2b_2$.

The corresponding amount of energy intake as food, I, is then calculated from Winberg,s (1956) energy equation

$$I = \frac{1}{\epsilon_a}(M + G) \quad (4)$$

where ϵ_a = assimilation efficiency, and $G = G_B + G_G$ = total amount of energy storage in the fish per time interval.

For situations between complete starvation and maintenance of body energy the model makes use of a parameter c, which is a dimensionless measure of the appropriate feeding level. At starvation the total metabolism, M*, can be expressed as

$$M^* = (b_1M_S + ((1-\epsilon_t)/\epsilon_t)G_G)/(1-a_2) \quad (5)$$

where ϵ_t = efficiency of energy transfer from body to gonad. If gonads continue to develop during starvation, the decrease in body energy under these circumstances, G_B^*, must meet requirements for metabolism and gonad growth so that

$$-G_B^* = M^* + G_G. \quad (6)$$

At maintenance the requirements for metabolism and gonad growth are met completely by intake and processing of food so that the maintenance food intake I'' is given by

$$I'' = \frac{1}{\epsilon_a}(M'' + G_G) \quad (7)$$

where M'' is calculated from equation 3b with $G_B=0$. At intermediate feeding levels, metabolism and food requirements are therefore

$$M = -G_B^* + c(\epsilon_a I'' + G_B^*) - G_G \quad (8)$$

and $$I = c\ I''. \quad (9)$$

Here c is found from the relationship $(1-c)=-G_B/G_B^*$, with $c=0$ at starvation and $c=1$ at maintenance.

Slightly different metabolic equations are used for eggs and larvae. In the case of eggs the energy for metabolism is provided by the decrease in yolk supply; the total decrease in egg energy during development is defined by a yolk utilization efficiency (Lasker 1962) so that the daily expenditure can be found once development time is known. For larvae a constant growth effciency is specified and food consumption is computed directly; metabolic expenditures are then determined from the energy equation.

Population processes — Output from the metabolic submodel for individual fish is combined with information on population structure to give age-class and population energy flows.

TABLE 1. Parameters used to define the initial state of the St. Margaret's Bay plaice population

		Value used		
		Females	Males	Source
Density — no. per ha		16.7	16.7	Bakken(1973)
(reference age-class)		(8)	(8)	
Annual mortality rate		0.40(1–4)	0.40(1–4)	" "
(age range in years)		0.13(4–12)	0.14(4–10)	
		0.51(12–24)	0.75(10–17)	
Age at first maturity — range in years		6–15	2–9	" "
Regression constants —				
1. length(cm)/age(y) $1=1_\infty(1-e^{-k(t+t_0)})$	1_∞	85.5	45.0	" "
	k	0.04	0.10	
2. weight(g)/length(cm)	t_0	1.23	0.98	
$w=c_1l^{c_2}$	c_1	0.0047[a]	0.0047[a]	
	c_2	3.099	3.099	
Regression constants —				
1. gonad wt(g)/body wt (g)	c_3	0.0252	0.020	MacKinnon(1972b)
$w_G = c_3\ w^{c_4}$	c_4	1.261	1.0	
2. fecundity/weight(g)	c_5	1094.0	–	Pitt(1964)
$f=c_5w^{c_6}$	c_6	0.901	–	
Energy per unit dry weight (cal/g) at spawning	body	900	990	MacKinnon(1972b)
	gonad	1430	613	

[a]Corrected from Bakken (1973) to give gutted weight at length on June 1.

Calculations with the model start at an appropriate reference date and evaluate energy flows and the changes in energy storage for successive time intervals until a year's cycle is completed. The reference date is the day following an idealized time when the population spawns. Although the initial state is defined in principle by the data listed in Table 1 the model makes use of various other properties, e.g. the total population energy per unit area, which are computed from these data.

A change in state of the population during an interval of time involves an alteration in its energy due to changes in the energy content per fish (e) and/or numbers (n) in each age-class. The energy per unit area, E, of the population can be expressed as

$$E = \sum_i n\ e \tag{10}$$

where i = the age-class. Changes in population energy during a time interval dt are then given by

$$\frac{dE}{dt} = \sum_i n \frac{de}{dt} + \sum_i e \frac{dn}{dt} \tag{11}$$

which implies

(change in population energy) = (production) – (elimination).

Calculations with the model are made for finite time intervals so that suitable average values of n and e are assigned for each age-class. The changes in numbers ($\triangle n$) and energy of individuals ($\triangle e$) during that interval are then evaluated, and the total energy change is computed according to equation 11. It should be noted that, for each iteration within a given year, changes in numbers must be zero or negative and hence the second term on the right in equation 11 is essentially always negative.

Changes in numbers per age-class are computed from numbers present at the start of a time interval and the mortality rate for the age-class. I have assumed that the mortality rate is constant throughout the year, with a value dependent upon he annual rate defined by the stable age distribution. Seasonal variations in mortality rate could be considered by making minor changes in the model. In the case of eggs, larvae and 0+ fish, however, different mortality rates are applied to each stage of development.

The change in energy of individuals during anp time interval is computed directly from data used to define the seasonal energy storage cycle and the net yearly increases in length and weight which are specified by weight/length and length/age regressions. Length changes occur only during a specified part of the year.

A steady state is assumed so that the net change in population energy over an annual cycle is zero. In formal terms, using equation 11,

$$\oint \frac{dE}{dt} = \oint \left(\sum_i n \frac{de}{dt} + \sum_i e \frac{dn}{dt} \right) = 0$$

so that

$$\oint \sum_i n \frac{de}{dt} = -\oint \sum_i e \frac{dn}{dt}. \tag{12}$$

The term on the left of equation 12 represents the net annual production of the population due to the change in energy of eggs, larvae, and older fish. The term on the right represents the removal (including yield) of energy from th epopulation by predators or other causes.

An important point to note is that the stable age assumption does not imply population production during a particular time interval ($\sum_i n \triangle e$) must equal elimination ($\sum_i e \triangle n$). In many temperate zone fish populations, for example, production takes place principally during summer months while elimination and energy expenditures for maintenance occur throughout the year. Also net annual production is not necessarily equal to annual elimination of a particular age-class; production exceeds elimination in younger age-classes and vice versa in older age-classes.

Production ($n\triangle e$) and elimination ($e\triangle n$) are computed with the model for each time interval and age-class and then summed to give annual population values. Since production and elimination per time interval are not in general equal, distlnction is made between gross annual production (including energy that is stored and used for population maintenance at other times of year) and net annual production which must equal annual elimination.

The proportion of elimination that constitutes yield can be determined from the ratio of fishing mortality to total mortality of the age-class.

Data and Parameters

Most of the information used in calculations with the model was obtained for the American plaice population of St. Margaret's Bay. A few parameter values were determined from information applying to other plaice populations or to fish in general. Data on the distribution, abundance, and population dynamics of plaice in the Bay were obtained in an extensive study during 1966–68 and the results are given in Bakken (1973). The entire Bay was surveyed with otter trawls which were calibrated for mesh selection and capture efficiency. An analysis of variance of numbers of fish in about 20 hauls at each of three localities was made to determine the error of sampling at a particular locality compared to the variation found among different areas. Approximately 3000 fish were tagged at each of three localities and the results, in accord with an analysis of vertebrae numbers, indicated little intermingling between plaice in the Bay and areas offshore. The densities of plaice estimated from tagging were in elose agreement with the values obtained from trawling. A length-frequency distribution (N=6957) based upon 22 samples from randomly chosen localities was used in estimating mortality rates. Measurements of age, from otolith readings, and length of about 530 fish were used to determine the annual growth of both sexes. Observations on the seasonal patterns of growth

and reproduction were also made. The idealized date of spawning of the Bay population was taken as May 31 so that the reference date for starting the production analysis was June 1. Spawning in this population has been observed from April to June and the peak period is generally in May.

Parameters used to define the initial state of the population are given in Table 1. Age 8 was chosen as the reference age-class because these fish were fully vulnerable to the net used (Bakken 1973) and they were relatively abundant in the research vessel catch. Annual mortality rates of vulnerable age-classes were estimated graphically from the length-frequency and age/length data of Bakken (1973). Since an increase in mortality rate with age is commonly found in unfished populations (Nikolsky 1969). two linear segments were used to represent the age-frequency data plotted on a graph with a semilog scale. On this basis the annual instantaneous mortality rates of females aged 4–12 and 12–24 were estimated at 0.13 and 0.51, respectively. This interpretation cannot be distinguished statistically from a constant mortality rate for all age-classes vulnerable to capture, but it seems to be more realistic. The mortality rate of fish aged 1–4, which are relatively invulnerable to capture, was estimated from the slope of the numbers/age regression (semilog plot) based upon the sample data of Bakken (1973). Mortality rates of larvae and 0+ fish were estimated by extrapolating forward from the total egg production, and backward from the numbers of older fish. The fecundity/weight regression of Pitt (1964) for plaice in the Newfoundland area was assumed to apply to the St. Margaret's Bay population.

Parameters and data required in addition to those needed to define the initial population state are presented in Table 2. Values for input variables of the model are generated from the data on seasonal variations in energy storage and standard metabolism of individual plaice.

The seasonal energy storage pattern of Bay plaice was computed from data on seasonal changes in weight relative to length (condition) and percent dry weight of materials, the annual gonad weight cycle and variations in the energy per unit dry weight of body and gonad materials (MacKinnon 1972b). Here I assumed that the annual length increase in Bay plaice occurred from June 1 to November 30, in accord with observations on plaice in the Newfoundland area (Pitt 1966). Standard metabolism was determined from laboratory observations on the low routine rate of oxygen consumption of plaice taken from the Bay at different seasons (MacKinnon 1973).

An estimate for the constant a_2 of the metabolic submodel (equation 1 etc.) has been made for plaice under laboratory conditions (D. Ware unpublished data) and, since no method of checking was available, the value of $a_2 = 0.067$ was applied to plaice in the Bay. A value for a_1 was chosen so that daily food intake computed with the model was approximately zero during winter months when the observed decrease in body energy is equal to the energy expenditures for

TABLE 2. Additional parameters and data used in applying the model to the St. Margaret's Bay plaice population.

		Value used	Source
Data:			
Seasonal energy storage patterns in individual fish, according to sex and state of maturity		time series	MacKinnon(1972b)
Seasonal patterns of standard metabolism		time series	MacKinnon(1973)
Parameters:			
Metabolic submodel:	a_1	0.3	see p. 1721[a]
	a_2	0.067	see p. 1721
	ϵ_p	0.65	see p. 1722
	ϵ_a	0.80	Winberg(1956)
	ϵ_t	0.90	see p. 1722
	K_y	0.65	see p. 1722
	K_1	0.50	see p. 1722
Iteration interval(days)		4	arbitrary
Time (days) from spawning to			
hatching		18	see p. 1722
active feeding		23	see p. 1722
metamorphosis		108	see p. 1722
Survival probabilities			see Table 3

[a]Rationale for choice of these parameter values is given in the text at the pages indicated.

standard and activity metabolism. The estimate of $a_1 = 0.3$ obtained by this method is close to that for laboratory conditions. A food processing efficiency of 65% was used, in accord with results of feeding experiments with American plaice (D. Ware unpublished data). Assimilation efficiency was taken as 80% (Winberg 1956). Although no information is available on the efficiency of energy transfer from body to ovary, a value of 90% was used, as suggested by biochemical considerations (Blaxter 1962). A yolk utilization efficiency (K_y) of 65% was used on the basis of information reviewed by Lasker (1962) on energy conversion in eggs. Net growth efficiency of larvae (K_1) was estimated at 50%, a value slightly higher than the maximum efficiency reported by Edwards et al. (1969) for the smallest 0+ European plaice.

Instantaneous changes in numbers per age-class of older fish are determined by the annual mortality rate values but additional information is required to describe the mortality pattern of eggs, larvae and 0+ fish. Values for the times (from June 1) to hatching, transition to active feeding, and metamorphosis were assigned on the basis of a review of information on larval plaice ecology (MacKinnon 1972a). Since data on mortality rates during these periods are very scarce for most marine fish and substantial year-to-year variations can be expected, the mortality pattern was described by various estimates of the probabilities of survival for each period (see Table 3 and Discussion).

Computer Program Description

A Fortran program for the model was written for execution on a Control Data 3150 Computer. The program comprises: (1) a preliminary section for data input and calculation, with printout, of properties defining the initial population state; (2) a main loop for calculations required for successive iterations, with provision for printout of information at particular times of year; (3) a final section for summation of production and energy flow values over all age-classes and time intervals, and then printout of these annual population properties.

In the main loop energy changes of individual fish of each age-class are evaluated from input data, and metabolic expenditures and food requirements are computed with the metabolism submodel. This information is then combined with changes in numbers during the time interval to give production, metabolism, food requirements, and elimination for each male and female age-class. These calculations are performed for fish of age 1 and up and finally, depending upon the time of year, the corresponding computations are made for eggs, larval or 0+ fish as appropriate. At the end of the loop the various production values and other quantities of interest are summed over all age-classes to give population values appropriate to the particular time interval.

Application to Plaice in St. Margaret's Bay

Initial Steady State

On June 1 the model gives an average total energy of 4.2 kcal/m² for plaice distributed over the depth range of 20–100 m in the Bay. Fish aged 1 and up constitute 4.02 kcal/m² and eggs the remaining 0.18 kcal/m². The biomass equivalents of this energy are 4.2 g/m² (41.6 kg/ha) and 0.12 g/m² for fish and eggs, respectively. Biomass per age-class ranges from 0.021 g/m² for fe-

Table 3. Production estimates computed with the model for larvae and 0+ fish of the St. Margaret's Bay plaice population.[a]

	larvae			0+ fish		
Survival probability:	"average"	high	low	"average"	high	low
Energy flows (kcal/m²year) —						
Ingestion	1.5	11.3	0.14	1.3	7.4	0.3
Metabolism	0.5	4.0	0.05	0.7	4.2	0.2
Production	0.5	4.0	0.05	0.3	1.6	0.07
Elimination	0.4	2.3	0.05	0.4	3.3	0.27
Average biomass (g/m²) —	0.12	1.1	0.01	0.07	0.28	0.02
Energy ratios —						
Production/Ingestion	0.35	0.35	0.35	0.2	0.2	0.2
Elimination/Production	0.8	0.6	0.9	1.3	2.0	3.8
Production/Biomass[b]	4.2	3.7	4.7	4.2	5.7	2.8

[a]Assuming 7.32×10^6 eggs/ha at spawning, 190 fish at age 1, and the following survival probabilities:

	"average"	high	low
egg: spawn/hatching	0.25	0.50	0.10
larvae: hatching/active feeding	0.25	0.50	0.10
larvae:active feeding/metamorphosis	0.03	0.10	0.01

[b]With conversion factor of 1 kcal/g.

males aged 1 to a peak of 0.29 g/m² at age 12; corresponding values for males are 0.030 g/m² at age 1 to 0.22 g/m² at age 10. Density at this time is 728 fish/ha (0.07/m²), overall sex ratio (males:females) is 0.96:1, and the number of eggs present is 7.3×10^6/ha.

Seasonal Patterns

Daily body growth rate, metabolism, and food consumption of individual fish attain peak values in summer and gross growth efficiencies of about 30% are estimated for this period (Fig. 2). During winter months food consumption decreases to zero, metabolic rate declines to a value close to the standard rate in the laboratory, body growth rate becomes negative, and growth efficiency is zero, or negative, depending upon one's definition. In the case of mature females gonad growth continues during winter months at the expense of body energy stores. The total metabolism during summer months is approximately three times the standard laboratory rate.

The curves shown in Fig. 2 represent the main seasonal features of energy conversion and storage in American plaice but several points should be noted concerning their interpretation. Given that any increase in length occurs only during a certain period in the year, the body growth rate curve is generated directly from observational data so that the detailed shape of the curve is greatly influenced by variability in these data. For population estimates fish of all age-classes were considered to have a similar seasonal pattern in spite of differences in absolute values of growth rate; this was necessary because data were available only for 30–40-cm females and 20–30-cm males.

Metabolism and food requirements are computed from growth data with the metabolic submodel. These values are affected by any uncertainty in parameter values used in the model. On the other hand, several checks on the reliability of these seasonal energy flow patterns are available. The period of negative growth rate is consistent with observations that food is virtually absent from the stomachs of plaice during that time of year (Powles 1965). For the winter period the observed rate of decrease in body energy is close to the energy requirements for metabolism and gonad maturation computed with the model; in this case the metabolism approaches the standard level, estimated independently from laboratory data, while gonad growth rate is derived directly from field data. During summer months, the estimated rates of food consumption and growth efficiencies are close to those observed in laboratory feeding experiments for American plaice (D. Ware unpublished data) and other flatfishes (Pandian 1970; Edwards et al. 1969) when food is relatively abundant.

The seasonal variations in production, metabolism, and food consumption of each age-class follow patterns similar to those for individuals in the particular age-class (Fig. 2). By contrast, elimination exhibits little seasonal variation because mortality rate is held constant. In August, for example, age 11 females ingest 38 kcal/ha per day while production is 13.6 kcal/ha per day and elimination is 1.2 kcal/ha per day. During March a metabolic expenditure of 6.4 kcal/ha per day plus gonad production of 0.7 kcal/ha per day is balanced by the decrease in body energy, while elimination is estimated at 1.0 kcal/ha per day.

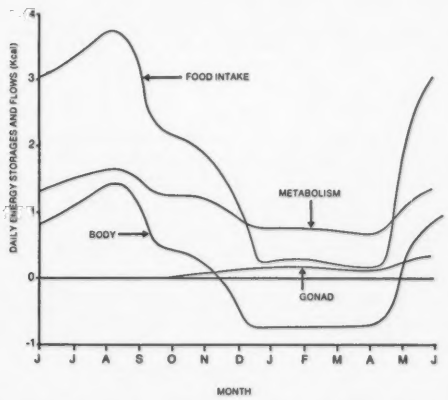

FIG. 2. Seasonal variation in daily body and gonad growth rate, metabolism and food intake of a mature age 11 American plaice (*Hippoglossoides platessoides*) female.

Age-Class Distributions

The model indicates that the distribution of population biomass among age-classes (Fig. 3A) has a peak value near the median age of the population. The absolute value of population biomass is directly proportional to the estimated population density whereas the distribution of biomass among age-classes is greatly influenced by the value for mortality rate. Note that I assumed a relatively higher mortality rate for older fish and that biomass of larger fish would be somewhat greater if a constant rate is used for all ages.

As might be expected, the values for metabolic expenditure and food consumption for each age-class vary directly with the biomass values and also have peak values near the median age of the population (Fig. 3A)

The distribution of production and elimination among age-classes (Figure 3B) is such that net production exceeds elimination up to about age 9 and is the reverse thereafter. In age 1 females, for example, the ratio of net production to elimination is 2.1 whereas in age 20 females the ratio is 0.33. This feature is an important requirement for the generation of an equilibrium biomass distribution with a constant rate of elimination.

The sharp peaks in the body production and elimination curves appear to result from the change to different constant mortality rates for various age-class ranges. These peaks are flattened somewhat if mortality rate is made to vary continuously with age-class but the production/elimination ratio of the younger age-classes remains greater than one. Egg production and the number of eggs spawned by a particular age-class are also directly

affected by the shape of the survival curve for mature fish.

Annual Population Estimates

Annual energy flows for the American plaice population in St. Margaret's Bay, including values associated with three different larval/0+fish mortality patterns, are presented in Fig. 4 and Table 3.

It appears that larvae and 0+ fish account for some 20% of the total population ingestion of 13.8 kcal/m² per year. The largest fraction of the population energy intake, 62%, is used for metabolic expenditures. Even though the summer period is 1.4 times the overwintering period, summer metabolism accounts for about 80% of total annual metabolism and winter metabolism for 20%. The main factor responsible for the relatively high summer metabolism is the heat released during food processing.

Net annual production by the population, 2.3 kcal/m², is 16.7% of annual ingestion. Larvae and 0+ fish together account for 34% of net production even though their biomass is only 4% of the population total. The gonad production of 0.2 kcal/m² per year is 8.6% of total population production or 13% of production by fish aged 1 and up. Production by the population during the summer period (i.e. gross production) is approximately twice the annual net production of fish aged 1 and up. Winter metabolic requirements amount to 49% of gross production; the quantity remaining is divided between net-body-production (44%) and gonad production (7%).

The net-body-production/biomass ratio for the whole population is 0.4 and the ecological efficiency (elimination/ingestion) is 17%. When larvae and 0+ fish are excluded from the calculations these figures become 0.3 and 12%, respectively.

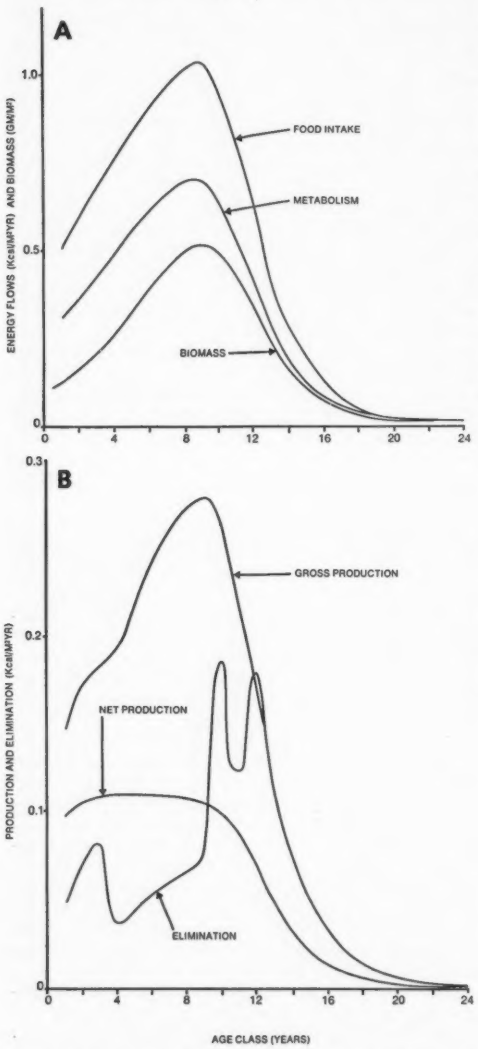

FIG. 3. Distribution among age-classes of A) biomass, food intake, and metabolic expenditure, and B) annual production and elimination for the American plaice (*H. platessoides*) population of St. Margaret's Bay, N.S.

Discussion

Model Parameter Values

Mortality rate parameters used in the model are derived by a combination of estimation and extrapolation from available data. The rate for the intermediate age range of fish vulnerable to capture by trawl agrees well with natural mortality estimates

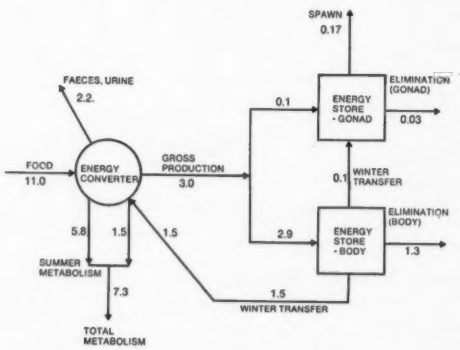

FIG. 4. Annual energy flows (kcal/m²) for the American plaice (*H. platessoides*) population (fish aged 1 and up) of St. Margaret's Bay, N.S.

for American plaice in other areas (Powles 1965; Pitt 1972) and for European plaice, *Pleuronectes platessa* (Beverton and Holt 1957). Changes of ±20% in the model values of 0.13 and 0.14 $year^{-1}$ for females and males, respectively, in this age range give net changes of about ±2% in population biomass and change of a similar amount in energy flows. Distributions of biomass and energy flow among age-classes are clearly also modified by a change in mortality rate. The higher rate assigned to older fish is close to the 50–75% annual mortality rate derived by Kennedy (1954) for an unexploited lake trout population in which the maximum age of about 25 years is similar to that of St. Margaret's Bay plaice. When this mortality rate is decreased by 20%, population biomass increases by 6%. The mortality rate used for fish aged 1–4 is based upon extrapolation beyond the effective range of the sampling gear so that model calculations were made for rates greater and less than the reference value of 0.40 $year^{-1}$. Whereas the predicted numbers of small fish are greatly affected by the choice of an age 1–4 mortality rate, the population biomass and energy flows change by only a few percent.

The mortality rate used in the model for plaice in their first year of life is similar to the decline in numbers by 4–5 orders of magnitude reported by Gulland (1965) for Arctic cod (*Boreogadus saida*) in their first year. For survival of haddock and cod eggs Gulland gave values of 5–17% whereas Kipling and Frost (1970) considered that 70% of pike eggs survived to hatching. A figure of 0.25 was therefore chosen as the normal probability of survival to hatching of a plaice egg, with 0.1 and 0.5 considered to represent extreme values. In view of Gulland's observation that 23–29% of "late" cod and haddock eggs survived to become larvae, the mean survival probability for plaice during this period was taken to be 0.25, with 0.1 and 0.5 as extreme values. Larval fish mortalities of 90–98% are common (Farriss 1960) so that for plaice a mean larval probability of survival to metamorphosis of 0.03 was chosen, with 0.1 and 0.01 as extreme values. Given these survival probabilities and the numbers of eggs and age 1 fish from Table 3, the mortality rates for 0+ fish are 6.1, 9.7, and 1.9 $year^{-1}$ for mean, high, and low larval mortalities, respectively. These values are comparable with those reported by Steele and Edwards (1970) for 0+ European plaice.

The energy flows computed for larvae and 0+ fish are affected by changes in the survival probability values used in the model. Biomass and ingestion of larvae, for example, change by an order of magnitude (Table 3) when either the high or low figures for survival probability are used. These extreme values for ingestion and their associated mortality patterns are unlikely to occur, however, if mechanisms associated with food availability have a significant effect on the patterns of growth and mortality. Steele and Edwards (1970) found that the effects of an increase in size of individual 0+ plaice were generally related to a decrease in numbers, such that a fairly uniform rate of energy intake in any 1 year resulted. Their estimated values for total intake in each of 4 years differed by less than a factor of two. Food limitations have also been found to affect the mortality patterns of perch (Menshutkin et al. 1968).

The estimated number of age 8 fish per unit area has a proportional effect on population biomass and energy flow values. Variations in the regression constants of, for example, the weight/length regression within the error range of their estimates have only a slight effect on model output. A similar conclusion applies to parameters of the metabolic submodel except the food processing efficiency which may vary with diet and activity (Warren 1971). The efficiency of 65% used in the model is within the range of values reported for fish. Kerr (1971) found that for trout the cost of food processing was 29% of the energy intake; assuming an assimilation efficiency of 80% this gives a food processing efficiency of 64%. Edwards et al. (1969) concluded that for 0+ European plaice amounts of food in excess of maintenance were converted to fish flesh with a maximum efficiency of 55%; when corrected for assimilation this implies a food processing efficiency of about 69%. Use of food processing efficiencies of 50 and 75% in the model gives changes in population metabolism of +37% and −16%, respectively.

Energy Flows and Efficiencies

The energy flow patterns and conversion efficies for the St. Margaret's Bay plaice population are of considerable interest because they apply to a relatively rare situation — an unexploited resident stock of an important commercial species.

Annual ingestion by plaice from benthic food resources, 12.3 $kcal/m^2$, is approximately half the estimated yearly production (25 $kcal/m^2$) of the invertebrate community in the deeper parts of the Bay. This production figure is based upon the calorific values of Brawn et al. (1968) and on biomass estimates derived from more extensive data (D. L. Peer unpublished data) than those reported in the paper. A production/biomass ratio of 3.1 was used, in accord with observations of Peer (1970) on *Pectinaria hyperborea* and the general considerations of Waters (1969). In the North Sea, where the type of bottom community (McIntyre

1961) appears to be similar to St. Margaret's Bay, Steele (1965) estimated macrobenthos production at 3 g C/m^2 per year or about 30 kcal/m^2 per year.

Most of the production by larger benthic animals in the deeper parts of the Bay appears to be grazed by fish rather than by invertebrates such as starfish. In addition to the consumption by plaice there is cropping by other demersal fish such as Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinis*) during certain seasons. By contrast, Thorson (see Hardy 1959) estimated that perhaps 98% of benthos potentially available as food for fish in the North Sea is actually consumed by invertebrates such as starfish. Thus Hardy concluded that fish yields might be substantially increased by destroying important invertebrate competitors. More recent evidence suggests, however, that this estimate of invertebrate consumption is too high and that demersal fish are probably the major consumers of benthos in the North Sea. From an extensive grab sample survey of the Central North Sea, Ursin (1960) estimated the average biomass of starfishes *Astropecten irregularis* and *Asterias rubens* at 1.4 g/m^2. Assuming a growth efficiency of 30% (Christensen 1970) together with a production/biomass ratio of 2, the annual consumption of these starfish is $1.4 \times 2/0.3 = 9.3$ g/m^2. Steele (1965) estimated food requirements for demersal fish in the North Sea at approximately 17 g/m^2 per year.

Production of demersal fish per unit area in St. Margaret's Bay appears to be similar to the North Sea. The estimated demersal fish yield for the North Sea is 1.7 kcal/m^2 per year (Gulland 1970) and production is expected to exceed this figure by at least a factor of two (Steele 1965). Net annual production for the benthos-consuming part of the Bay plaice population is 1.6 kcal/m^2 and, since plaice appear to consume about half of the benthic invertebrate production, the production of demersal fish in the Bay may well amount to twice the estimate for plaice.

Comparison of production efficiencies for plaice with those for other fish populations is complicated by uncertainties associated with the inclusion or otherwise of larvae and 0+ fish in the estimates. Even with larvae included the production/biomass ratio of 0.4 for plaice is low but the value is close to that expected for a population dominated by large, slow-growing fish (Mann 1969). An ecological efficiency of 12% for that part of the population consuming benthos is within the 5–15% range appropriate to a variety of populations (Odum 1971) but with the inclusion of larvae a relatively high value of 17% is obtained. Gonad production amounts to 13% of net production by plaice aged 1 and up and is comparable with the figure of 9.5% found by Mann (1965) for fish older than 1 year in a river community. When revised estimates for 0+ production (Mann et al 1972) are considered however, gonads account for 3.2% of the total freshwater fish production whereas 8.5% of total population production is used for gonads in plaice.

Calculations of potential fish yields (e.g. Ryther 1969) often consider that about half of the total production is available to fishing. However, Mann et al. (1972) observed that most production of freshwater fish populations occurs in the youngest stages and not more than a quarter of the total production is available for harvesting by man. Since plaice is a commercial marine species an estimate of the relative amount of production by fish vulnerable to capture is of some practical interest. With Bay plaice of age 7 (length $\sim$ 24 cm) onwards considered to be vulnerable to fishing, the elimination of 0.33 kcal/m^2 per year for age classes 1–6 may be added to that for larvae (0.42 kcal/m^2 per year) and 0+ fish (0.35 kcal/m^2 per year) to give a total of 1.10 kcal/m^2 per year which is available to predators other than man. This amounts to 51% of the elimination by all age-classes of the population so that 49% of elimination, less an amount for natural mortality of older fish, represents the potential fish yield.

Seasonal variations in the ingestion and other energy flows of fish populations are of both practical and theoretical importance. Given this information, one can calculate in energy terms the increase in yield that can be obtained by cropping fish at the end of the feeding season rather than taking the same number of fish at the end of the overwintering period. While this strategy for obtaining higher yields has long been appreciated, quantitative estimates have apparently not been made until now. From a theoretical point of view it is of interest to establish whether seasonal cycles are essential to the function of the plaice population as a component of the Bay ecosystem. As a result of investigations with an aquatic ecosystem model, Winberg and Anisimov (1969) suggested that inherent cyclic phenomena may be necessary features for a stable and productive ecosystem. The information for plaice could be used in an appropriate ecosystem model to test this hypothesis.

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NOTES

The Skate, *Raja richardsoni* Garrick 1961, Assigned to *Bathyraja*

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TEMPLEMAN, WILFRED. 1973. The skate, *Raja richardsoni* Garrick 1961, assigned to *Bathyraja*. J. Fish. Res. Board Can. 30: 1729–1732.

The deepwater skate, *Raja richardsoni* Garrick 1961, is assigned to the genus *Bathyraja* on the basis of studies of 18 specimens from the northwest Atlantic including 10 mature males. It is similar to other *Bathyraja* species and distinguished from *Raja* and *Breviraja* species especially by having in the clasper tip a ventral terminal cartilage completely enclosed within the skin of the ventral lobe and lacking a free sharp edge, and a sentinel (accessory terminal cartilage no. 1) in the form of a hatchet-shaped knife with a sharp distal edge. Its clasper also has a projection, whose support is the distal process of the ventral marginal cartilage, always found in *Bathyraja* but not in *Raja* of the northeast Atlantic. It differs also from *Raja* in having an uncalcified or little calcified anterior part of the rostral cartilage, in the freedom of the appendices of the rostrum from the shaft and in the more anterior extension of the pectoral radials toward the snout.

TEMPLEMAN, WILFRED. 1973. The skate, *Raja richardsoni* Garrick 1961, assigned to *Bathyraja*. J. Fish. Res. Board Can. 30: 1729–1732.

L'auteur assigne la raie des profondeurs, *Raja richardsoni* Garrick 1961, au genre *Bathyraja*, se basant sur l'étude de 18 spécimens, dont 10 mâles mûrs, provenant du nord-ouest de l'Atlantique.

Cette raie ressemble aux autres espèces de *Bathyraja* et diffère de celles des genres *Raja* et *Breviraja* surtout par la présence, au bout de la myxoptérygie, d'un cartilage terminal ventral complètement enveloppé dans la peau du lobe ventral et dépourvu d'un bord libre tranchant et d'une sentinelle (cartilage terminal accessoire no. 1) ressemblant à un couteau en forme de hachette à bord distal tranchant. Ses myxoptérygies possèdent également une sorte d'ergot dont la cartilage est le procès distal du cartilage marginal que possèdent toutes les espèces de *Bathyraja* mais non celles de *Raja* du nord-est de l'Atlantique. Cette raie diffère également de *Raja* en ceci qu'elle possède à l'avant du cartilage rostral une partie non calcifiée ou très peu calcifiée, les appendices sont dégagés du corps du rostre et les radiaux des pectorales se prolongent davantage vers l'avant en direction du museau.

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THIS study on 18 specimens including 10 mature males from the northwest Atlantic showed that the deepwater skate, *Raja richardsoni* Garrick 1961, should be assigned to *Bathyraja*. Comparisons were made with *Raja* and *Breviraja* species and two species of the North Atlantic (*Raja spinicauda* Jensen 1914 and *Breviraja pallida* Forster 1967) considered to belong to *Bathyraja* (sensu Stehmann 1970). The main studies were of the claspers and the clasper cartilages, the rostrum and its appendices, the cranial fontanelles, and the numbers of vertebrae and pectoral radials.

Raja richardsoni was described from a single mature female (Garrick 1961). The claspers of a male specimen examined by Stehmann (1970) were dried up and unsuitable for detailed study. Stehmann tentatively included the species in his *spinicauda* group of *Bathyraja* and later in his paper noted the possibility that it represents a subgenus of *Bathyraja*.

Materials and methods — The *richardsoni* specimens studied were obtained from the northwest Atlantic (Templeman 1973).

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Vertebrae and pectoral radials were counted on radiographs. In counting pectoral radials, the anterior and posterior extensions of the basal pectoral cartilages were counted as radials. (This is also Stehmann's (correspondence March 1973) practice.) For the radiographs of pectoral radials, the pelvic fin was prevented from overlapping with the posterior part of the pectoral.

To determine the anteriormost predorsal caudal vertebra, I used two methods. These methods usually produced different truncal and predorsal caudal counts, but provided the same total counts for the two parts of the vertebral column studied.

The first method of counting the vertebrae followed modifications of the methods of Hubbs and Ishiyama (1968), Krefft (1968), and G. Krefft and M. Stehmann (correspondence January–March 1973). For this method, vertebral columns were dissected in the region of the first haemal arch and a pin inserted into the vertebral column vertically, touching the ventral median anterior edge of the first haemal arch. A vertical pin was also inserted at the origin of the first dorsal fin. Care was taken to ensure that the vertebral column was horizontal dorsoventrally when the vertical pins were inserted. On the radiograph, the posteriormost predorsal caudal vertebra was that touched by the pin at the origin of the first dorsal fin if at least 50% of its length was ahead of the pin at its point of insertion into the vertebra; otherwise the count began at the next anterior vertebra. The anteriormost predorsal caudal vertebra was that touched by the pin at the beginning of the first haemal arch if at least 50% of this vertebra was posterior to the pin at its point of insertion into the vertebra; otherwise the next posterior vertebra was counted as the first predorsal caudal. (The first haemal arch appeared to begin near the anterior part of the centrum of its vertebra (Fig. 4B).) The remaining vertebrae extending anteriorly to and including the most anterior complete centrum embedded in the vertebral plate (Fig. 4D) were included as truncal (abdominal or precaudal) vertebrae. As far as possible, the radiographs, especially of the tail region, were taken in lateral view.

The other method followed that of Krefft (1968) in designating as the anteriormost predorsal caudal vertebra the first obviously shorter diplospondylous vertebra following the longer monospondylous vertebrae of the truncal region (Ishiyama 1952, 1958; Springer and Garrick 1964; Hubbs and Ishiyama 1960; Krefft 1968).

Monospondylous vertebrae occasionally occur among the diplospondylous vertebrae near the beginning of the haemal arch region (Fig. 4C). These interpolated monospondylous vertebrae were counted as part of the predorsal caudal group. Also, between the obviously monospondylous and the obviously diplospondylous vertebrae, there often occur one or occasionally two vertebrae so intermediate in size as to be difficult to assign to the anterior long or the posterior short group of vertebrae. I included these intermediate vertebrae with the truncal vertebrae. Ishiyama (1952, fig. 2A) apparently counted the vertebra of intermediate length with the predorsal caudal group, whereas Springer and Garrick (1964) included it with the truncal vertebrae.

In the predorsal caudal but not in the truncal region, four of seven *richardsoni* from the northwest Atlantic had two to four vertebrae imperfect in size or shape but recognizable as individual vertebral centra. For counts of vertebrae, each of these imperfect vertebrae was taken as one (Templeman 1970).

Comparisons of skeletal and related characteristics — Claspers — The clasper shape and structure and the clasper cartilages of *richardsoni* (Fig. 1 and 2) are generally similar to those of *Bathyraja spinicauda* of Stehmann (1970) and of *Bathyraja smithii* of Hulley (1970, 1972) and in external form to those of *B. pallida* of Stehmann (1970). Consequently only structures showing considerable differences or needing elucidation are described.

Dorsally on the outer side, the large dorsal terminal cartilage no. 1 (dT_1) has an anterior long triangular extension, not so evident nor angular in *B. spinicauda*. The dT_2 in *richardsoni* and *B. spinicauda* is so very firmly attached to the distal end of the dorsal marginal cartilage (dM) as to appear to be part of it, and it is thus labelled dM by Stehmann (1970) for *B. spinicauda*. I have followed Ishiyama (1958, fig. 6, 9, 12) in identifying the dT_2. Also, this cartilage in a preserved clasper of *richardsoni* became detached from the dM in 10% NaOH solution. A dT_3 as identified by Ishiyama (1958), is present, firmly inserted into the dT_2 and called dT_2 by Stehmann for *B. spinicauda*. A dT_4 is also present, firmly attached by connective tissue to the dT_3, and being presumably related to the projecting point on the distal right of the cartilage of *B. spinicauda* called dT_2 by Stehmann (1970, plate 8).

The deep, obliquely longitudinal slit lies between the dT_2 laterally and the distal process of the dM medially. In some but not all mature claspers of *richardsoni*, there is a second, considerably smaller slit immediately distal to that previously described, with a shallow pocket extending anteriorly underneath the tip of the distal process of the dM. This minor slit is separated by a bridge of tissue from the more proximal longer and deeper slit. The anterior pointed portion of the ventral terminal cartilage (vT) is relatively longer and more pointed in *richardsoni* than in *B. spinicauda*.

The *richardsoni* specimens agree with *Bathyraja* of Ishiyama and Hubbs (1968) and differ from *Raja* and *Breviraja* of the same authors in lacking a large shield (vT) with a free sharp edge along its ventral border and in having the vT completely enclosed within the skin. Also, in *richardsoni*, as in *B. spinicauda* and *B. pallida* (Stehmann 1970) and some Pacific species of *Bathyraja* and *Rhinoraja* but not of *Raja* (Ishiyama 1958, 1967), the sentinel, whose support is the accessory terminal cartilage no. 1 (aT_1), is a hatchet-shaped knife with a sharp distal edge. In *richardsoni*, the spike (aT_2) is lacking.

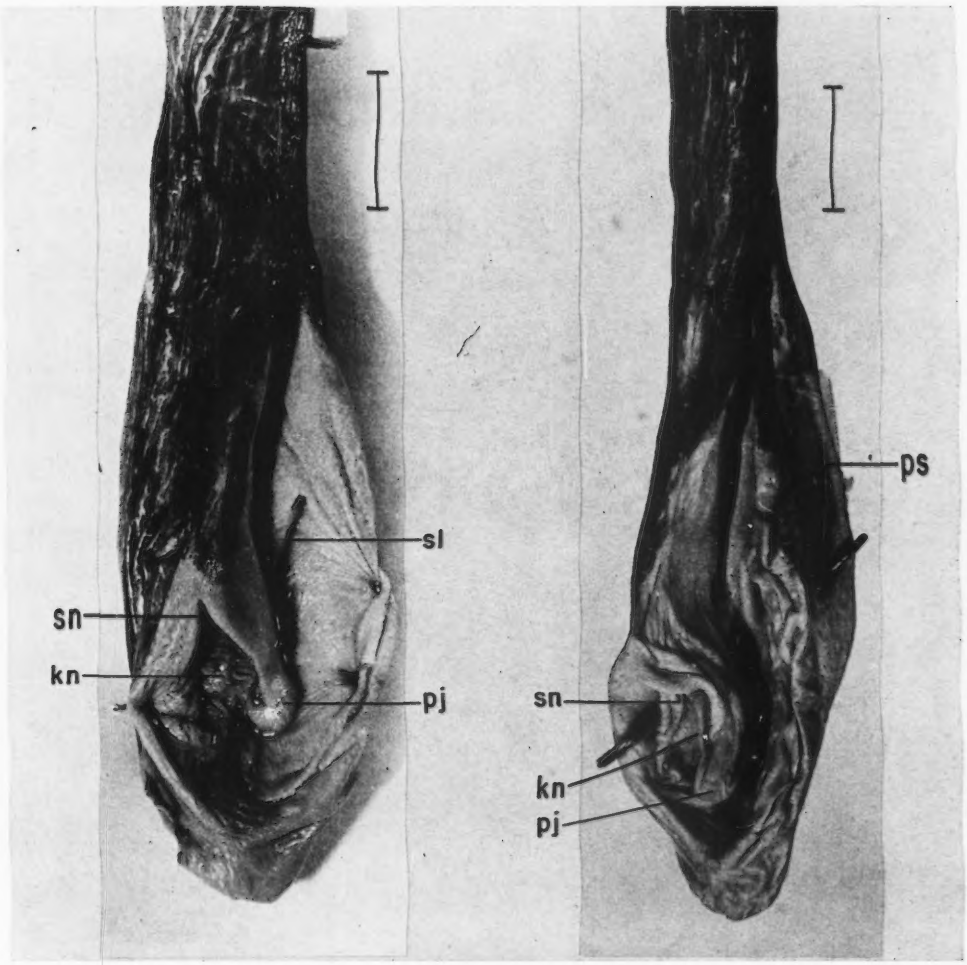

FIG. 1. Clasper of *richardsoni*. Ventral (left) and ventrolateral (right). views of distal part of left clasper of mature male to show: pseudosiphon (ps), slit (sl), sentina (sn), knife (kn), and projection (pj). (The indicator lines for magnification in this and all other figures represent 20 mm.)

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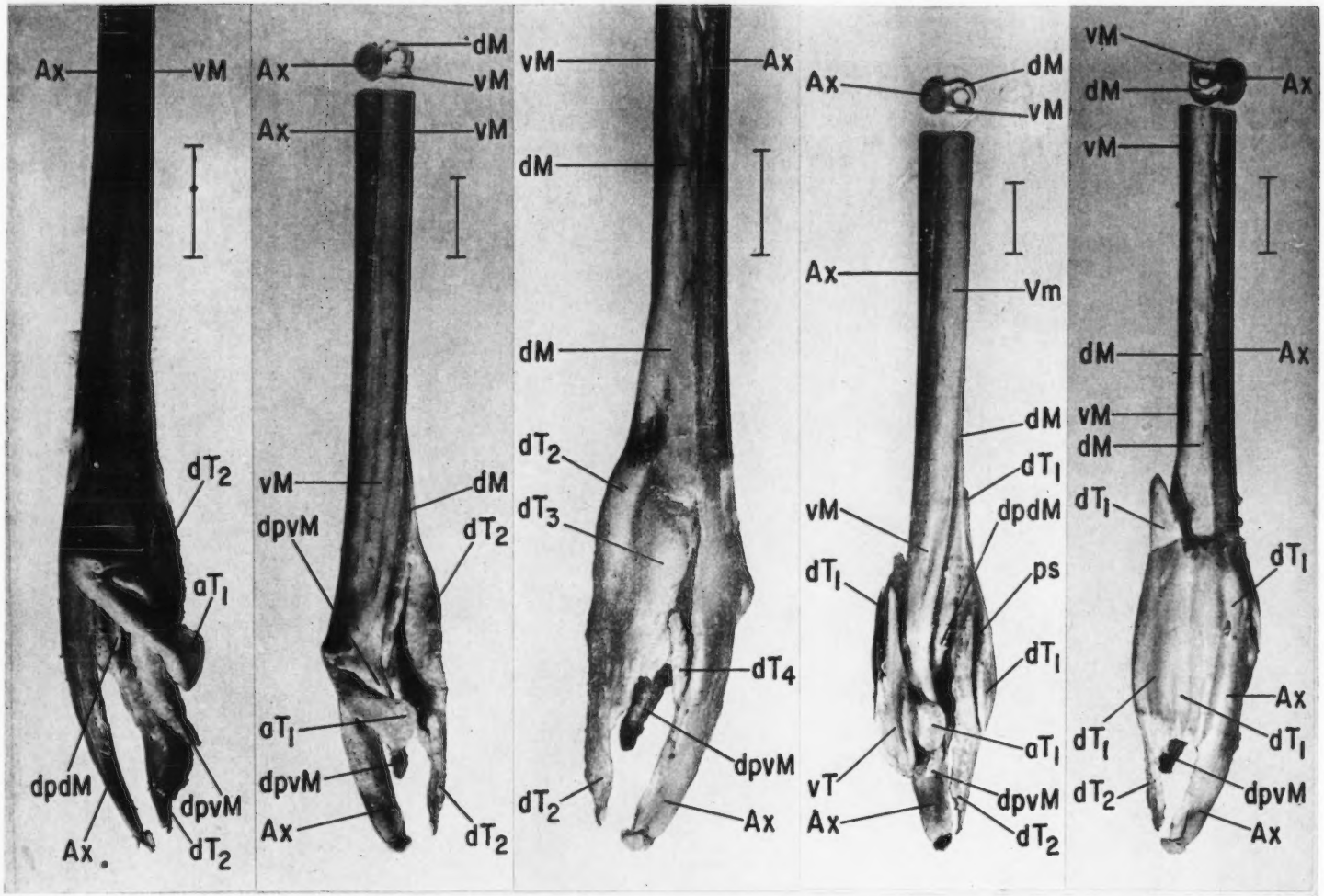

FIG. 2. Cartilages of distal part of left clasper of mature *richardsoni* male showing, from left to right: ventral, ventrolateral and dorsal views with the dT_1 and the vT removed; and ventral and dorsal views with these cartilages in position to show axial (Ax), dorsal marginal (dM), ventral marginal (vM), distal process of dorsal marginal (dpdM), distal process of ventral marginal (dpvM), dorsal terminals No. 1, 2, 3, 4 (dT_1, dT_2, dT_3, dT_4,), accessory terminal No. 1 (aT_1), ventral terminal (vT), and also the pseudosiphon (ps).

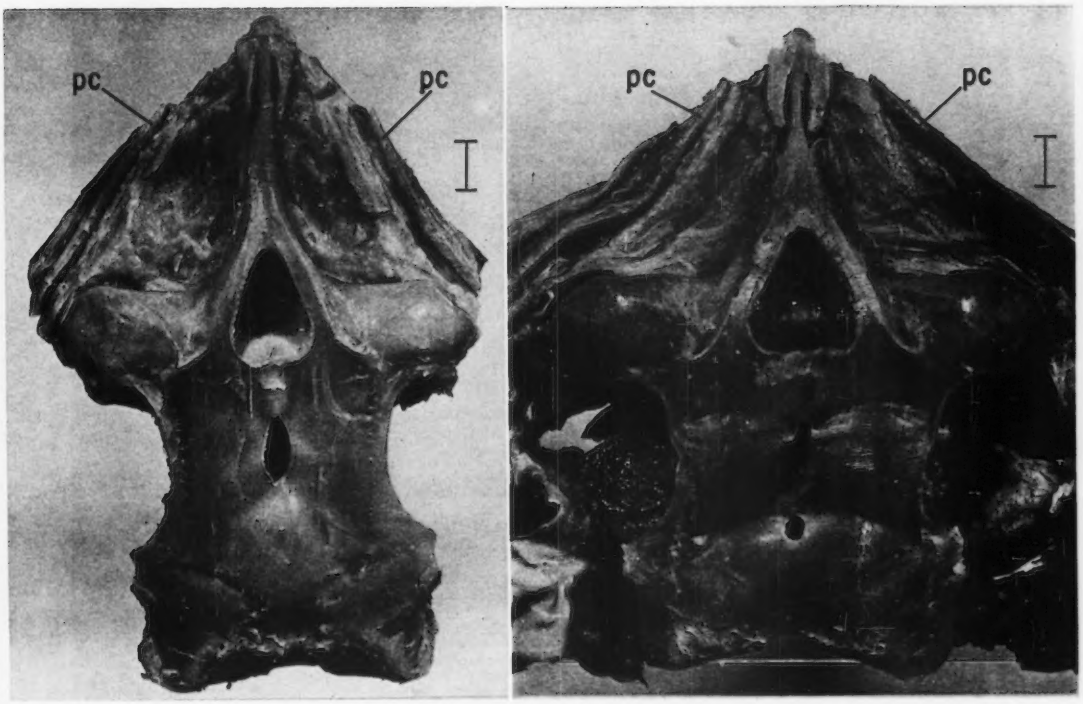

FIG. 3. Crania of *richardsoni* to show: large anterior supracranial fontanelle at base of rostrum, 1 smaller posterior fontanelle at left and 2 at right, also rostrum and its anterior wing-like appendices and forward extensions of the pectoral cartilages (pc).

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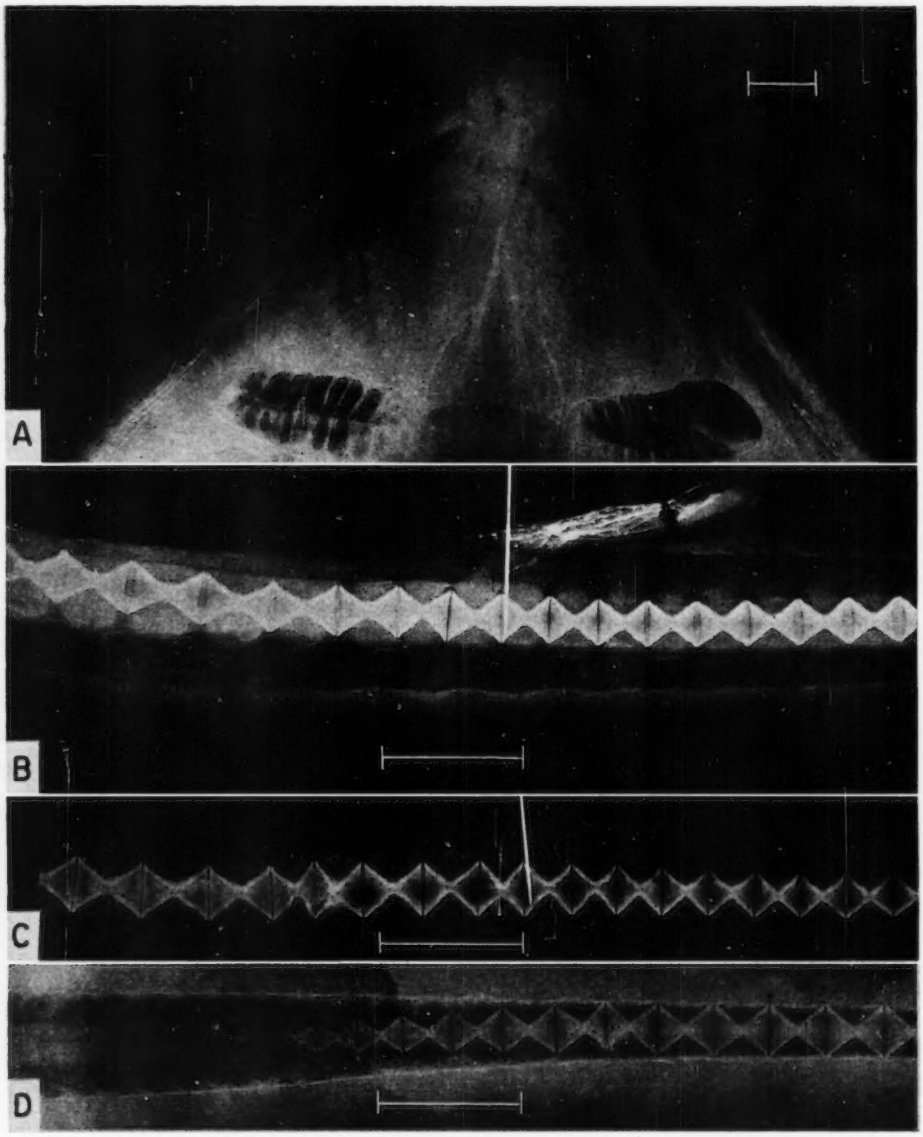

FIG. 4. Radiographs of *richardsoni* A, snout showing, centrally at base, lines of calcification outlining anterior fontanelle and leading forward triangularly into a canal in base of rostrum; also, anterior extension of pectoral radials and basal pectoral cartilage on each side. B, lateral view, ventral side upward, showing vertical pin at median anterior beginning of first haemal arch, long monospondylous vertebrae anteriorly to the left and short diplospondylous vertebrae posteriorly beginning 2 vertebrae anterior to the pin. C, lateral view, ventral surface upward, showing pin at beginning of first haemal arch with short vertebrae beginning 5 vertebrae anterior to (left of) the pin and with 2 intervening long vertebrae posteriorly before the short vertebrae begin again. D, the anteriormost vertebrae embedded in vertebral plate. The anteriormost half vertebra (left) was not counted.

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It is present in all northeast Atlantic species of *Raja* but not in *Bathyraja spinicauda* or *B. pallida* (Stehmann 1970). However, Ishiyama (1958, fig. 11) shows a small aT_2 in three species of *Breviraja* (now *Bathyraja*) (Ishiyama and Hubbs 1968) from the Japanese region. Also, *richardsoni* has a long distal process of the vM, the cartilage of the projection of Stehmann (1970), found in all North Atlantic species of *Bathyraja* and not in *Raja* of the northeast Atlantic. Also, in *richardsoni* as in other North Atlantic species of *Bathyraja* there is a large and deep pseudosiphon lying ventrolaterally, medially to the dT_1, and said by Ishiyama and Hubbs (1968) to be found in most species of *Bathyraja* but not in *Breviraja* or *Raja*. A pseudosiphon, however, is present in many North Atlantic species of *Raja* (Stehmann 1970).

Rostrum, anterior extensions of pectoral cartilages, and cranial fontanelles — The *richardsoni* specimens agree with *Bathyraja* of Ishiyama and Hubbs (1968) in that the anterior part of the rostral cartilage is bird-like, flexible, and apparently calcified little or not at all (Fig. 3). It is so little calcified that it does not appear in radiographs of the whole snout (Fig. 4A). The wing-like appendices of the rostrum are free posteriorly from the shaft of the rostrum, thus differing from *Raja* of Ishiyama (1958). Also, in the form of the anterior angle of each appendix, the junction of the appendix with the rostrum, and the head-like anterior extension, they resemble *Bathyraja* and differ from *Breviraja* of Ishiyama and Hubbs (1968).

The shaft of the rostrum has the broad base typical of *Bathyraja*. But because the snout is short, although the rostrum narrows considerably, it does not become as slender anteriorly as in the longer-snouted species *B. spinicauda* (see Stehmann 1970). *Bathyraja pallida*, with a snout shorter than that of *B. spinicauda* but longer than that of *richardsoni* in terms of disc length, appears from the abbreviated diagram in Forster (1967) to be intermediate in the thickness of the anterior part of the shaft of the rostrum between *richardsoni* and *B. spinicauda*. Similarly, the Japanese species of *Bathyraja* (*Breviraja* in Ishiyama 1958, fig. 28) show a considerable variety in rostral form from the relatively short, very broad-based, and moderately thick rostrum of *B. matsubarai* to the very slender and long rostrum of *B. trachouros*.

Bathyraja of Ishiyama and Hubbs (1968) agrees with *Breviraja* in having the pectoral radials extended forward to near the appendices of the rostrum near the tip of the snout. In contrast, in the *Raja* group the pectoral radials are much farther from the snout (Ishiyama 1958; Bigelow and Schroeder 1948, 1953, 1965).

The anterior extensions of the pectoral cartilages (Fig. 3 and 4A) differ in the two specimens of *richardsoni* dissected, one extending to opposite the posterior parts of the rostral appendices (Fig. 3 left) and the other to about one third from the anterior ends of the appendices (Fig. 3 right). The anterior tips of the pectoral cartilages thus extend well forward in relation to the rostral appendages and the snout, not as far as in the long-snouted, weak-rostrummed species *Bathyraja spinicauda* but about as far as in *B. pallida* of Forster (1967) and considerably farther than in *Raja* of Ishiyama (1958, fig. 27).

The *richardsoni* specimens have the broad-based and relatively short anterior form of supracranial fontanelle found in most species of *Bathyraja*, because of the broad, triangular shape of the base of the rostrum, in contrast to the more elongate form of anterior fontanelle common in *Raja*. Similarly, the posterior fontanelle or fontanelles are narrow, as is more common in *Bathyraja* than in *Raja* of Ishiyama (1958, fig. 28, 27).

The large anterior fontanelle in the base of the rostrum and in the anterior part of the cranium is similar in position to that in *B. spinicauda* of Stehmann (1970) but triangular rather than boat shaped. The posterior fontanelles are differently shaped and different in number in the two examined (Fig. 3). Such variability of fontanelle number and shape is not unusual in skates, as illustrated by Stehmann (1970) for several *Raja* species.

Vertebral and pectoral radial numbers — For seven western North Atlantic specimens of *richardsoni*, the ranges of my vertebral counts (averages in parentheses) on the basis of pin markers (Fig. 4) were: truncal, 38–43 (40.1); predorsal caudal, 63–69 (65.4); total truncal and predorsal caudal, 103–109 (105.6) By Krefft's method, the values were: truncal, 34–39 (37.1); and predorsal caudal, 64–71 (68.4). Totals for the two methods are the same. For pectoral radials of six western North Atlantic specimens, the ranges (averages) were: left, 81–88 (85.3), and right, 82–88 (85.7), the left and right counts being similar in four and differing by one in two specimens.

It is evident, as Stehmann (1970) indicated, that vertebral and pectoral radial numbers are not useful for separating *Raja* and *Bathyraja* genera of the Atlantic even though vertebral numbers are useful (Ishiyama 1958) in Japanese skates. For 19 *Raja* species from the eastern North Atlantic, Stehmann (1970), using Krefft's method, found the number of truncal vertebrae to range from 25 to 44 and that of predorsal caudal vertebrae from 42 to 81. For *Bathyraja pallida*, *richardsoni*, and *Bathyraja spini-*

cauda these ranges were 33, 33–35, 36–38 and 66–67, 64–69, 77–82, the totals for *richardsoni* (correspondence Stehmann March 1973) being 97 and 104. In the species of *Raja*, Stehmann (1970) found the pectoral radials to range from 59 to 105 and, for small numbers of *Bathyraja pallida*, *richardsoni*, and *Bathyraja spinicauda*, the ranges were 79–80, 77–82 and 95–98.

Conclusions — Richardsoni is not a species of *Raja* or of *Breviraja*. Its claspers in their essential elements definitely resemble those of *Bathyraja*, as do also the form, flexibility, and lack of calcification of the anterior tip of the rostrum and of its appendices, the relationship of the anterior extension of the pectoral cartilages to the snout, and the forms of the base of the rostrum and of the anterior fontanelle. In its clasper form and elements it is very close to *B. spinicauda* (and also close to *B. pallida* in external clasper elements but the clasper cartilages of this species have not been studied). In the anterior part of its rostrum, the anterior extension of the pectoral cartilages, and vertebral and pectoral radial numbers, it is closer to *B. pallida*, which has relative snout and tail lengths intermediate between those of *richardsoni* and *B. spinicauda*.

Within the present genera, *richardsoni* is obviously a species of *Bathyraja*. Clasper elements are fundamental to the separation of this genus from *Breviraja* and *Raja*. Further breakdown of *Bathyraja* into subgenera must await a wide-ranging study as it is apparent from Ishiyama (1958), Stehmann (1970), and the present paper that much greater differences in the form of clasper elements occur in Pacific than in Atlantic species of *Bathyraja*.

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Occurrence of DDT Residues in Beluga Whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T.

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ADDISON, R. F., AND P. F. BRODIE. 1973. Occurrence of DDT residues in beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T. J. Fish. Res. Board Can. 30: 1733–1736.

Tissues of Beluga whales (*Delphinapterus leucas*) sampled from a transient herd off the Mackenzie Delta, N.W.T., contained residues of the DDT pesticide group. Muscle and liver contained *p,p'*-DDE and *p,p'*-DDT; total residue concentrations in these tissues were approximately 0.01 and 0.02 ppm fresh weight, respectively. Blubber contained *p,p'*-DDE, *p,p'*-DDT, and *o,p'*-DDT; total residue content was approximately 2–4 ppm fresh weight.

ADDISON, R. F., AND P. F. BRODIE. 1973. Occurrence of DDT residues in beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T. J. Fish. Res. Board Can. 30: 1733–1736.

Des tissus de béluga (*Delphinapterus leucas*) prélevés à même un troupeau de passage au large du delta du Mackenzie, T.N.-O., contenaient des résidus des pesticides du groupe DDT. Le muscle et le foie contenaient *p,p'*-DDE et *p,p'*-DDT; la concentration totale des résidus dans ces tissus était approximativement 0.01 et 0.02 ppm de poids frais respectivement. La graisse contenait *p,p'*-DDE, *p,p'*-DDT et *o,p'*-DDT; le contenu en résidus totaux était d'environ 2–4 ppm de poids frais.

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THERE is little information about the distribution of organochlorine pesticide residues in Canadian arctic biota. In a survey of residue levels in freshwater fish, Reinke et al. (1972) found that samples from the Northwest Territories generally contained lower residue concentrations than those from southern Manitoba or Ontario. Holden (1970) reported lower residue concentrations in blubber of ringed seals (*Pusa hispida*) from Baffin Is. (in the Eastern Arctic) than in gray (*Halichoerus grypus*) or harp seals (*Pagophilus groenlandicus*) from the Gulf of St. Lawrence. Although species and other differences among the organisms analyzed hamper direct comparison of data from different geographical locations, it seems that the Arctic is less contaminated by organochlorine pesticides than our more densely populated areas.

We report here the results of some analyses of tissues of beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T. Although comparable samples were not available from other areas, nor was there comparable data in the literature, this population is of special interest since its migratory movements are believed to be limited to the Beaufort and Chukchi Seas (Sergeant and Brodie 1969). Residue concentrations observed in it may therefore reflect the level of pesticide contamination of the marine environment in this area.

Experimental — Beluga are hunted by the native people during the summer months to provide fresh and preserved food. Samples were taken from adults of this catch during July 1972. Seven beluga whales were sampled at Shallow Bay (68°55′ 135°20′) and another seven at Kugmallit Bay (69°25′, 133°50′). These locations are about 50 miles apart in the Mackenzie Delta, N.W.T. Samples of blubber and muscle from the middorsal region, and of liver, were taken from the Shallow Bay group, and blubber samples only were taken from the Kugmallit Bay group. The Shallow Bay samples were preserved in 10% formalin and stored in glass jars, and the Kugmallit Bay samples were kept on ice, and subsequently frozen. All samples were shipped to Dartmouth and analyzed within 6 months of collection.

Ten grams of tissue were dissected from the interior of the sample, and extracted by homogenising with the acetonitrile-based system described by Onley (1964). All solvents were reagent grade, redistilled from glass. Aliquots of the extracts were evaporated to dryness for gravimetric lipid determination, and further aliquots were cleaned up by chromatography on Florisil and analyzed by gas–liquid chromatography, substantially as described previously (Addison et al. 1972). Selected samples were analyzed on a 4-foot × ¼-inch (ID) glass

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column packed with 10% EGSS-X on 100–120 mesh Gas Chrom Q (preconditioned: Applied Science Labs. Inc.) operated at 195 C.

Results and discussion — Analytical data are presented in Table 1. Blubber lipid content was in the range expected (Kleinenberg et al. 1964) but no data referring specifically to beluga liver or muscle lipid were available. However, Ackman et al. (1965) have reported a comparable liver lipid content of 3% in the fin whale (*Balaenoptera physalus*). The observed beluga muscle lipid content (about 1% fresh weight of tissue) seems reasonable.

Only residues of the DDT group of pesticides were confidently detected. Treatment of the 6% ether–hexane eluate from Florisil with alcoholic KOH (Holden and Marsden 1967) confirmed the presence of *p,p'*-DDT[1] through conversion to *p,p'*-DDE, and treatment of selected dehydrochlorinated samples with CrO_3 (Miles 1972) demonstrated the presence of *p,p'*-DDE through oxidation to *p,p'*-dichlorobenzophenone. A peak co-chromatographing with *p,p'*-DDD on both polar and nonpolar columns was observed, but was too small to be reliably measured. (French and Jeffries (1971) reported that prolonged storage of avian liver by freezing or preservation in formalin was accompanied by degradation of *p,p'*-DDT to *p,p'*-DDD, probably through bacterial activity but total DDT group levels (ΣDDT) were not affected. (No such degradation was observed in the present work.) No peaks resembling the polychlorinated biphenyl (PCB) mixtures available to us (Aroclors: Monsanto, Ltd.) were detected in the dehydrochlorinated or oxidised samples. (Minimum detectable concentration of PCBs would have been approximately 0.5 ppm lipid.) No peak attributable confidently to dieldrin could be detected in the 15% ether–hexane eluate from Florisil.

ΣDDT residues in liver and muscle represented 0.02 and 0.01 ppm of tissue wet weight, respectively, and in blubber, 2–4 ppm wet weight of tissue. (Although preservation of tissues in formalin may result in some dehydration, the error involved in estimating residue concentrations on a wet weight basis is relatively small (less than 10%: French and Jeffries 1971), particularly in tissues rich in lipid.) Residue concentrations in lipid were fairly similar in liver and muscle, but in blubber were about twice as high. The Shallow Bay blubber samples had slightly higher residue levels (on a lipid basis) than the Kugmallit Bay samples, but the difference was significant only in the case of *p,p'*-DDE ($P < 0.05$ by t test). In general, residue levels were below those found in a Hudson Bay beluga blubber oil (Addison et al. 1972) and were well below those observed in marine mammals from areas in Eastern Canada (Holden and Marsden 1967; Gaskin et al. 1971) but were higher than those reported in Pacific gray and sperm whales (Wolman and Wilson 1970). However, direct comparison of data is complicated by the many factors which may influence residue accumulation (Addison et al. 1973).

[1]The following abbreviations are used in this note: *p,p'*-DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; *p,p'*-DDE, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene; *p,p'*-DDD, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane; *o,p'*-DDT, 2-*o*-chlorophenyl-2-*p*-chlorophenyl-1,1,1-trichloroethane; *o,p'*-DDE, 2-*o*-chlorophenyl-2-*p*-chlorophenyl-1,1-dichloroethylene.

TABLE 1. Lipid, and DDT concentration in lipid, from tissues of beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T.

Tissue & location	No. of samples	% Lipid	(Mean ± s.d.) *p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	*o,p'*-DDT	ΣDDT
Liver (Shallow Bay)	7	2.93±0.82	0.56±0.18	tr[a]	0.21±0.06	ND[b]	0.78±0.23
Muscle (Shallow Bay)	7	1.14±0.29	0.68±0.21	tr	0.23±0.09	ND	0.91±0.26
Blubber (Shallow Bay)	7	92.7 ±1.89	1.33±0.33	tr	1.27±0.34	1.27±0.34	3.90±0.89
Blubber (Kugmallit Bay)	7	94.5 ±2.20	0.77±0.47	tr	0.96±0.53	0.83±0.55	2.56±1.46

[a]tr — Trace.
[b]ND — Not detected.

The restriction of appreciable amounts of *o,p'*-DDT to samples of blubber tissue was unexpected. Its presence was confirmed by comparison of chromatographic behavior with that of standard on both polar and nonpolar columns, and by dehydrochlorination to *o,p'*-DDE (identified on the nonpolar column). Its occurrence in blubber, but not in other tissue samples, is difficult to explain. Selective accumulation by blubber seems unlikely, as does selective metabolism by liver or muscle. We might speculate that, following uptake from diet, residues are deposited first in blubber before mobilization to other tissues; the restriction of *o,p'*-DDT to blubber tissue might then indicate relatively recent uptake.

Beluga (1500–2000 animals) enter the delta estuary immediately after break-up of the shore ice and remain there, divided into several groups, through July into August accompanied by newborn. The benefit of this summer estuarine habitat is considered to be thermal, since water temperatures may reach 18 C in the shallow areas. Such temperatures would reduce the heat loss of the newborn calves and be energetically conservative for the nursing mothers (Sergeant and Brodie 1969; Brodie 1971).

There was no evidence of July feeding in the estuary and all the animals sampled had completely empty stomachs.

Since the beluga population from which these samples were taken is believed not to migrate beyond the Beaufort and Chukchi Seas (Sergeant and Brodie 1969), we conclude that their residues were accumulated from sources in the area at lower trophic levels, such as shrimp, worms, squids, and small fish (Vladykov 1946). Several routes of entry of pesticide residues into the Western Arctic environment can be envisaged. One of them is residues from local use; DDT has been used for biting fly control in the area, although on a limited scale. In and around Armed Forces bases, for example, DDT was applied at the rate of about 0.22 lb/acre during the 1950s and 60s (L. A. O. Roadhouse, personal communication). A further contribution might arise from drainage and spring inundation of areas similarly sprayed for pest control by the many small river based communities in the Mackenzie system. Atmospheric transport of DDT from areas of more intensive use might also contribute (cf. Abbott et al. 1965). Although winds in the area (Inuvik) are predominantly from the NW and NE (Atmospheric Environment Service Wind Summaries: J. F. Amirault, personal communication) occasional southerly winds might transport residues from spraying operations in upstream communities, particularly during May, June, and July. Finally, migration of components forming the diet of our samples from more contaminated areas might be considered, though to our knowledge, no detailed information exists about residue levels in such organisms. It is therefore not surprising that organochlorine residues were present in our samples. However, it is worth emphasizing that as far as we can reliably compare residue content between species, our arctic samples contained generally lower residue levels than those reported in marine mammals from more obviously contaminated areas.

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Karyotypes and Erythrocyte Sizes of some Diploid and Triploid Fishes of the Genus *Poeciliopsis*[1]

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CIMINO, M. C. 1973. Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus *Poeciliopsis*. J. Fish. Res. Board Can. 30: 1736–1737.

Karyotypes of diploid (2n=48) and triploid (3n=72) members of a unisexual–bisexual complex of fish of the viviparous genus *Poeciliopsis* consist of morphologically uniform acrocentrics, graded in size from large to small, and thus unsuitable for tracing evolutionary pathways within the complex. A comparison of blood samples from diploid and triploid forms showed that the mean diameters of erythrocytes of the triploids are one and one-half times those of diploids. This relationship provides an important tool for easily distinguishing the two types in samples taken from field populations.

CIMINO, M. C. 1973. Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus *Poeciliopsis*. J. Fish. Res. Board Can. 30: 1736–1737.

Les caryotypes des membres diploïdes (2n=48) et triploïdes (3n=72) d'un complexe unisexué–bisexué de poissons vivipares du genre *Poeciliopsis* consistent en acrocentriques morphologiquement uniformes formant une séri econtinue de taille décroissante et sont, pour cette raison, inappropriés pour tracer une lignée évolutionnaire. En comparant la formule sanguine des souches diploïdes et triploïdes, on constate que le diamètre moyen des érythrocytes des souches triploïdes est une fois et demie plus grand que celui des diploïdes. C'est un moyen simple et pratique de distinguer ces souches.

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A unisexual–bisexual complex of fishes of the genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes) consists of five forms. Two of these are gonochoristic (bisexual) species, *P. monacha* Miller and *P. lucida* Miller. The other three forms are unisexual, producing only female offspring. One of these, *P. monacha-lucida*, is diploid, and the other two, *P. monacha*-2 *lucida* and *P.* 2 *monacha-lucida*, are triploid (Schultz 1969). Karyotypes of these five forms were examined for evidence that the origin of the three unisexuals is hybridization of the two associated gonochoristic forms.

The number of chromosome sets of a cell is often reflected in cellular and nuclear size (Fankhauser 1945). Erythrocyte diameters were analyzed to detect the existence of this relationship in *Poeciliopsis*. Utilization of such a relationship would simplify studies of the relative abundance of the two ploidy levels in nature.

[1]This research was part of a thesis submitted in partial fulfillment of the requirements for the doctoral degree at the University of Connecticut.

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Materials and methods — The fish were laboratory stocks descended from fish caught in the Rio del Fuerte in Sonora, Mexico, in 1961 and 1968, and reared in greenhouses. For chromosome preparations, fish were injected with colchicine (McPhail and Jones 1966). After 2.5–3 hr, gill arches and ovaries were treated hypotonically with distilled water, fixed in two changes of methyl-Carnoy (3:1 absolute methanol:glacial acetic acid), and smeared by rubbing lightly over the surface of a cold, wet slide. Slides were stained with aceto-orcein and mounted in Diaphane.

For cell sizes, blood smears were prepared by conventional means and stained with Wright's stain (Gray 1964), using blood obtained from: (1) the wound caused by pithing, (2) the stump of the severed caudal peduncle, and/or (3) the excised heart. Slides were examined dry under a 40× objective. Circular cells were selected and measured with a calibrated eyepiece filar micrometer. About 100 cells each for three individuals of each of the five forms were measured. Differences in means were compared with student's t-test and 99% confidence limits were calculated (Sokal and Rohlf 1969, p. 374, p. 146). An overall mean and standard deviation, weighted for the sample size and variance of each subset (p. 178), was calculated for each of the five forms.

Results and discussion — Karyotypes derived from air-dried spreads stained with aceto-orcein cannot be used to distinguish between members of the complex, nor to trace the evolutionary origin of the unisexuals back to their gonochoristic progenitors. The karyotypes of each member consisted of a continuous series of acrocentric chromosomes ranging in size from 0.57 to 2.60 μ. No heterochromatin, secondary constrictions or other morphological features were apparent. The modal counts for the diploids, 2n=48, and for the triploids, 3n=72, verify previous reports of the chromosome numbers in *Poeciliopsis*, which were based upon the squash technique (Schultz 1969; Cimino and Schultz 1970).

Erythrocyte diameters of each triploid fish exceeded those of each diploid ($P=.001$). Although the ranges overlapped, means and confidence intervals for the different forms were distinct. The weighted means, standard deviations, and 99% confidence intervals in microns, were:

species		mean and SD	99% confidence limits
lucida	2n	7.35+/−0.39	7.30–7.41
monacha-1 *lucida*	3n	8.61+/−0.44	8.55–8.68
monacha-lucida	2n	7.33+/−0.35	7.28–7.38
2 *monacha-lucida*	3n	8.74+/−0.48	8.67–8.81
monacha	2n	7.42+/−0.45	7.35–7.49

Assuming that the triploids and diploids are similar in thickness of the cell — an assumption that is at least approximately correct for the conditions of preparation — the ratio of surface areas is 1.4. This corresponds to the 1.39 ratio of areas obtained from the data of Cherfas (1966) on triploid and diploid goldfish, and agrees closely with the 1.5 ratio expected for volumes.

Measurement of the erythrocyte diameters will prove a valuable tool for distinguishing between ploidy levels in natural populations. Blood smears can be easily made in the field, then stained and examined later. This technique will apply also to diploid and triploid *Poecilia* (Rasch et al. 1970), and diploid, triploid, and tetraploid ginbuna, *Carassius auratus langsdorfii* (Kobayasi et al. 1970). In the *Poeciliopsis* complex, the sympatric diploid *P. monacha-lucida* and triploid *P. monacha*-2 *lucida* are difficult to reliably distinguish in the field on the basis of morphology. Measurement of erythrocyte diameters is the only method presently available to quickly identify the large numbers of individuals necessary to determine their frequencies in natural populations.

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Identification of Fungal Encrustation on the Shell of the Snow Crab (*Chionoecetes bairdi*)

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VAN HYNING, J. M., AND A. M. SCARBOROUGH. 1973. Identification of fungal encrustation on the shell of the snow crab (*Chionoecetes bairdi*). J. Fish. Res. Board Can. 30: 1738–1739.

A black encrustment commonly found on the exoskeleton of snow crabs (*Chionoecetes bairdi*) from the Gulf of Alaska has been identified as the microfungus *Phoma fimeti*. It appears unrelated to other described crustacean shell diseases.

VAN HYNING, J. M., AND A. M. SCARBOROUGH. 1973. Identification of fungal encrustation on the shell of the snow crab (*Chionoecetes bairdi*). J. Fish. Res. Board Can. 30: 1738–1739.

Une incrustation noire communément observée sur l'exosquelette du crabe des neiges (*Chionoecetes bairdi*) du golfe d'Alaska a été identifiée comme étant le microfongus *Phoma fimeti*. Ce microorganisme ne semble associé à aucune autre maladie connue de la carapace des crustacés.

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SNOW crabs (*Chionoecetes bairdi*) taken along the coastline of the Gulf of Alaska frequently have a black encrustment on their exoskeletons. This condition has been termed "shell syndrome" (Brown 1971), presumably in reference to the shell diseases of other crustaceans.

Both fungi and bacteria have been implicated in European and North American shell diseases, respectively. Rosen (1970) states: "In all probability, the microbial community occupying the necrotic lesions is composed of both bacteria and fungi." Sindermann and Rosenfield (1967) describe "rust disease" of king crabs (*Paralithodes* sp.) in Alaska, and show a chitin-destroying bacteria to be the causative factor. This disease is characterized by a progressive darkening and softening of the exoskeleton, particularly on the ventral surfaces. Sindermann (1970) considers the shell disease of the American lobster (*Homarus americanus*) to be bacterial in etiology. This disease is characterized by pitting and sculpturing of the exoskeleton; initial lesions with white outer margins occur on the walking legs. Another disease of lobsters is termed "molting disease" and is characterized by yellowish splotches in an otherwise dark green exoskeleton. Sindermann considers the causative organism a chytrid fungus.

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Superficially, the condition on snow crabs does not resemble other shell diseases as described in the literature, generally brown, necrotic lesions on the exoskeleton. Macroscopically, the condition on snow crabs can be described as a mat of black, encrusting nodules or pustules forming a dense, hard, almost tar-like covering over parts of the exoskeleton (Fig. 1). Most commonly found over the posterior part of the carapace, it can, in extreme cases, extend anteriorally over the entire carapace and into the eyes and mouth parts. Ventrally, it can be found frequently on the posterior walking legs, but it appears less prevalent ventrally than dorsally. The black encrustment can be noticed on the abdominal flap, but we have never observed it in the female egg mass, as with the fungus *Lagenidium* sp. on the Atlantic blue carb (*Callinectes sapidus*), nor in any of the internal organs. Scraping the affected parts of the shell reveals a very slight pitting of the surface.

The junior author took scrapings from the carapaces of four heavily infected *C. bairdi* with a sterile scapel and transferred them to petri plates containing potato dextrose agar. When the isolate was in pure culture, it was transferred to POA tube slants. Based on cultural and morphological characteristics, the microfungus was identified as *Phoma fimeti* Brun., and identification was confirmed by the Central-bureau for Schimmel Cultures,

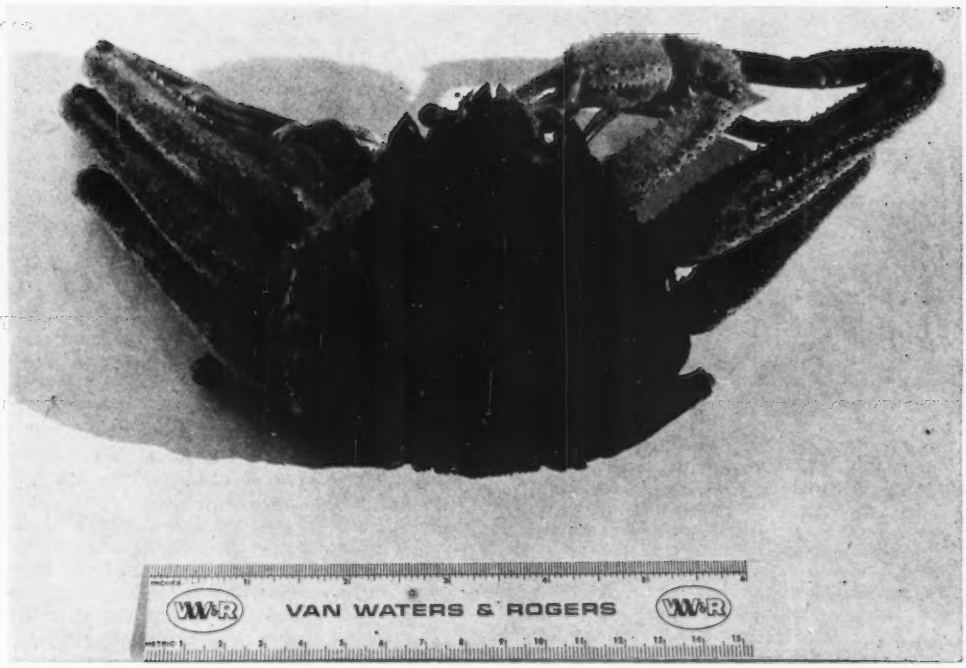

FIG. 1. Snow crab (*Chionoecetes bairdi*) with an extreme fungus encrustation.

Van Hyning and Scarborough — J. Fish. Res. Board Can.

Baarn, Netherlands. It is conceivable that the fungus identified is a secondary invader after initial bacterial destruction of the chitin, but we did not explore this possibility. No attempt was made to reinfect organisms from cultured material.

Johnson and Sparrow (1961) list *Phoma* sp. as being encountered often in marine and estuarine environments and existing usually saprophytically on wood or aquatic plants. No mention is made of occurrence on animals although two species occur among fouling organisms on the hulls of ships. Likewise, workers on crustacean diseases do not list *Phoma* sp. as being implicated in any described condition. *Phoma* sp. shows a marked tolerance to salinity and temperature. Where infected snow crabs are commonly encountered in Prince William Sound at a water depth of 150 m, the temperature is usually between 2 and 7 C and salinity around 32 ppt.

Phoma fimeti, as described by Dorenbosch (1970), is typically a soil-borne fungus, originally described from sheep dung and has also been isolated from paint, wood, and dead plant tissue. Species of *Phoma* are often isolated from Alaskan soils, but to our knowledge *P. fimeti* has not been previously reported.

This particular fungus appears to be species specific. Dungeness crabs (*Cancer magister*) and king crabs from areas with a heavy infection on *C. bairdi* show no gross encrustations. It also may be area specific; a comprehensive review of the Canadian Atlantic snow crab fishery by Watson (1969) and others makes no mention of a shell disease in *C. opilio*, and likewise the Japanese literature available does not mention this phenomenon. Pereyra (1968) does not record it on *C. tanneri* off Oregon and Washington. This lack of observations does not exclude its presence but suggests that, if present, it does not approach the distinctive characteristic features so noticeable on crabs in the Kodiak–Cordova areas of Alaska.

The syndrome does not have an apparently deleterious effect on the crab. In an unusually extreme case, the eyes and mouth parts of females that have undergone their terminal molt could be damaged and early death could result. Canneries will occasionally not process a load of badly infected crabs as the black nodules become mixed with the meat and are difficult to remove. Fishermen are aware of certain areas that seem to contain more syndrome than others. Brown (1971) sampled 1000 snow crabs from commercial catches around Kodiak Island and the Shumagin Islands. The average incidence of black nodules was 37%, varying from 7 to 75% in different areas. In May 1972, 731 snow crabs from various parts of Prince William Sound were examined. Eight percent had fungus encrustments, with less than 1% categorized as heavy or approaching the condition shown in Fig. 1.

As pointed out by the authors cited, there is a paucity of information on associations of fungi with marine crustaceans, and, as Johnson (1970) states: "At best the information is very general, but it does suggest that a host of biological problems exist in fungus–animal relationships — problems that await critical attention." Further confirmation of the relationship of a typically lignicolous fungi group existing saprophytically on a deepwater marine crustacean may be a case in point. Further work needs to be done in particular on positive confirmation of identification and to determine if other fungi or bacteria might be involved in this rather unique situation.

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Influence of Environmental Experience on Response of Yearling Rainbow Trout (*Salmo gairdneri*) to a Black and White Substrate

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Yearling rainbow trout (*Salmo garidneri*) selected black substrate regardless of size or rearing experience when first introduced into an experimental tank offering a choice of black or white. Differences in the degree of black selection among the various lots of the same strain during the initial 120 min of exposure, under an illumination of 10^{-2} lx, were correlated only with the level of swimming activity. By 24 hr, only pond-rearsd fish continued to select black while laboratory-reared fish were randomly distributed over black and white. The continuing wariness of only the pond-reared fish reveals the long-term effect of prior experience on juvenile trout behavior. This finding indicates the possible feasibility of environmental conditioning for wariness in the artificial propagation of hatchery-reared fish for live release.

RITTER, J. A., AND H. R. MACCRIMMON. 1973. Influence of environmental experience on response of yearling rainbow trout (*Salmo gairdneri*) to a black and white substrate. J. Fish. Res. Board Can. 30: 1740–1742.

Des truites arc-en-ciel (*Salmo gairdneri*) de moins d'un an choisissent un substrat noir, indépendamment de la taille ou de l'expérience acquise au cours de l'élevage, lorsqu'elles sont placées pour la première fois dans un bassin expérimental offrant un choix de blanc ou de noir. Les différences dans le degré de sélection du noir parmi les divers lots de la même lignée duraut les 120 premières minutes d'exposition, sous une illumination de 10^{-2} lx, n'ont de rapport qu'avec le niveau d'activité. Après 24 h, seuls les poissons élevés en étang continuent de choisir le noir, alors que les poissons élevés au laboratoire sont répartis au hasard sur le blanc et sur le noir. Une méfiance continuelle de la part seulement des poissons élevés en étang démontre l'effet à long terme d'une expérience préalable sur le comportement des jeunes truites. Cette constatation laisse entrevoir la possibilité d'un conditionnement environnemental de méfiance qui aurait son application dans l'élevage artificiel en pisciculture pour fins de peuplements.

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THE experiment described in this report is one of a series designed to determine the ecological significance of light to fish. Results of previous experiments in the series with rainbow trout (*Salmo gairdneri*) have shown that light intensity influences the activity of the fish, the fish's use of overhead cover, and its response to a black and white substrate (Kwain and MacCrimmon 1967, 1969a, and 1969b; MacCrimmon and Kwain 1966).

Previous experimentation by Kwain and MacCrimmon (1969a) revealed that domestic rainbow trout, when held under an overhead light with an intensity of 10^{-2} lx and exposed to a black and white background, began to select black at an age of 14 months. The authors questioned if this behavioral change was a function of age or was related to growth, i.e. size of fish. The present set of experiments was designed to determine if the observed differences in response to the black and white background had been influenced by the size of the fish. Also, trout from two rearing environments were observed to determine if rearing history influenced this behavioral response.

Materials and methods — Rainbow trout (*S. gairdneri*) of a fall spawning domestic strain were reared from the eyed-egg stage in black fiberglass troughs in the University Fisheries Laboratory until 5½ months old. The stock was then divided into two groups, one group being trans-

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ferred to two outdoor earthen ponds, the other being held in the laboratory in white concrete tanks at an overhead illumination of 50 lx. After an additional 6 months, the pond fish were returned to the laboratory and held for a further 2 months under the rearing conditions previously established for the laboratory group. The two groups were divided into four lots according to total length: small (7.2 ± 0.3 cm) and intermediate (13.8 ± 0.4 cm) laboratory fish, and intermediate (13.8 ± 0.2 cm) and large (21.4 ± 0.3 cm) pond fish. Because of the more rapid growth rate in the pond environment, neither small pond nor large laboratory fish were available. All fish were fed a dry commercial diet at rates recommended by the supplier. Test fish were fed to repletion 1 hr prior to being introduced into the experimental tank.

Fish were observed in a circular tank 1.83 m in diameter, holding water at a depth of 15 cm. Overhead illumination of 10^{-2} lx (energy equivalent of 0.004 $\mu W/cm^2$) was produced by diffused incandescent lights located 2.1 m directly above the water (Kwain and MacCrimmon 1969a). The bottom of the tank was divided into eight pie-shaped sections, alternately painted black and white.

The first set of 12 observations was begun 10 min after introduction of fish into the tank and continued for 120 min, and the second set was begun 24 hr later. For each observation a swimming activity index (Ritter 1970) and the number of fish over the black and white areas were recorded. The index was based on the number of fish moving during a 60-sec observation period and the pattern of fish movement. A high index value reflects a high level of activity.

Data for fish distribution were analyzed by the Chi-square method and corrected by combining probabilities from significance tests (Fisher 1963). A statistical esti-

TABLE 1. Distribution over a black and white substrate and indexes of swimming activity for four lots (20 fish in each of three replicates) of yearling rainbow trout (*Salmo gairdneri*) divided according to total fish length and rearing experience. Fishwere observed under an illumination of 10^{-2} lx during 120-min periods both immediately after and 24 hr after their introduction into the tank.

Test fish		Percent over black	Activity index[a]	Correlation coefficient[b]
		Immediately after introduction		
Laboratory				
Small	(7.2±0.3 cm)	63.0	0.76	−0.34[d]
Intermediate	(13.8±0.4 cm)	72.5[c]	0.58	−0.30
Average		67.8	0.67	−0.41[d]
Pond				
Intermediate	(13.8±0.2 cm)	80.0[c]	0.64	−0.68[d]
Large	(21.4±0.3 cm)	63.0	0.86	−0.56[d]
Average		71.5[c]	0.75	−0.69[d]
		After 24 hr		
Laboratory				
Small	(7.2±0.3 cm)	58.0	0.75	−0.20
Intermediate	(13.8±0.4 cm)	51.5	0.53	+0.45[d]
Average		54.8	0.68	+0.27[d]
Pond				
Intermediate	(13.8±0.2 cm)	85.5[c]	0.32	−0.72[d]
Large	(21.4±0.3 cm)	71.0[c]	0.58	−0.93[d]
Average		78.3[c]	0.45	−0.86[d]

[a]Based on the number of fish moving during a 60-sec observation period and the pattern of fish movement (Ritter 1970). A high index reflects a high level of activity.

[b]Correlation coefficient for the linear relationship between fish distribution over black and activity index.

[c]Difference between percentages over black and white areas significant at $P < 0.05$.

[d]Correlation between fish distribution over black and fish activity significant at $P < 0.05$.

mate of the dependence of fish distribution on activity was determined by linear regression analysis. Variations in the number of fish over black bottom areas with fish size and rearing experience were tested for significance by an analysis of variance (Fisher 1963).

Results and discussion — Immediately after introduction into the experimental tank all four lots selected, to varying degrees, the black rather than the white areas of substrate (Table 1). Observed variations in the degree of selection of the black areas among the four lots were correlated with differences in swimming activity rather than with the length of the fish or the type of environment in which the fish had been reared.

The selection of black areas by all lots of trout during the initial 120-min period after their introduction into the experimental tank was lower than that previously recorded by Kwain and MacCrimmon (1969a) but followed a similar behavioral pattern. This selection of black is interpreted as being a unique component of a complex behavioral response termed wariness by other researchers (Moyle 1969; Vincent 1960) and, in this case, a fright stimulus was provided by stress imposed on the fish during transfer from holding to experimental tanks and by the unfamiliarity of the fish with the test chamber. A similar use of overhead cover has been previously described by MacCrimmon and Kwain (1966).

After the test lots of fish had been in the experimental tank for 24 hr, differences in the response to the black and white bottom had become visually apparent and differences in the selection of black between lots with different rearing experience were *statistically significant* ($P < 0.05$). *Differences in* preference for the black areas between lots of fish, divided according to length within the laboratory and pond-reared groups, were not significant ($P > 0.05$). These results indicate, therefore, that the observed behavioral response is influenced by the environmental history of the fish rather than by their size. Thus it may be concluded that the changes in preference for black substrate among juvenile rainbow trout grown in the laboratory over many months as previously recorded by Kwain and MacCrimmon (1966a) were a function of age rather than size, and answers a question posed by these authors.

Of considerable significance is the finding that environmental experience modified the behavioral response of the same strain of rainbow trout when exposed to different sets of conditions for a 6-month period although reared under identical conditions for the first 5 months of life. Even after being returned to the laboratory condition for a 2-month period, the pond-reared fish retained a pronounced wariness (as expressed by the prolonged selection of black) not exhibited by the laboratory-reared lots.

The potentials of ecological conditioning in the artificial propagation of trout for live release has received negligible attention despite the obvious advantage of releasing fish best adapted for existence in various types of habitat. Results of the present study indicate the possible feasibility of use of environmental conditioning in production of hatchery-reared fish in order to enhance their probability of survival on release to natural waters.

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Endrin Uptake and Release by Fingerling Channel Catfish (*Ictalurus punctatus*)

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Fingerling channel catfish (*Ictalurus punctatus*) offered diets containing 0.04–4.0 μg endrin/g food (dry weight) rapidly accumulated the pesticide. Amounts in the tissues were directly proportional to amounts in the food. Dietary endrin had no measurable effect on growth or mortality. After endrin was withdrawn from the diet, it rapidly disappeared and could not be detected after 41 days. In fish exposed to 0.5 μg endrin/liter of water, mortalities began at tissue levels of 0.7 μg endrin/g of wet whole fish and total mortality occurred at levels near 1.0 μg/g.

ARGYLE, R. L., G. C. WILLIAMS, AND H. K. DUPREE. 1973. Endrin uptake and release by fingerling channel catfish (*Ictalurus punctatus*). J. Fish. Res. Board Can. 30: 1743–1744.

Des alevins de barbue de rivière (*Ictalurus punctatus*) soumis à des régimes contenant 0.04–4.0 μg d'endrine/g de nourriture emmagasinent le pesticide rapidement. Les quantités dans les tissus sont directement proportionnelles à celles de la nourriture. L'endrine dans la nourriture n'a pas d'effet appréciable sur la croissance ou la mortalité. Une fois soustraite du régime, l'endrine disparaît rapidement des tissus et ne peut être décelée après 41 jours. Chez des poissons exposés à 0.5 μg d'endrine par litre d'eau, les mortalités commencent à se produire à des niveaux tissulaires de 0.7 μg d'endrine/g de poids humide du poisson entier et la mortalité totale survient à des niveaux approchant 1.0 μg/g.

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FISH ponds are often constructed on land which has been under cultivation or receives runoff from cultivated fields. Pesticides retained in the soil may become available to fish through the food chain or directly from the water. Little information is available concerning chronic, low-level exposure to pesticides in food or water. We investigated the effects of endrin in feed and in water on the growth and survival of fingerling channel catfish.

Materials and methods — The research was conducted in two parts: channel catfish held in aquaria were fed purified diets containing endrin; other channel catfish fingerlings held in aluminum troughs were exposed to endrin in water.

We fed channel catfish fingerlings five agar-bound, purified diets that contained 4.0, 0.4, 0.04, 0.004, and 0.0004 μg endrin/g of dry feed. The nutritionally complete diets, in which corn oil was the endrin carrier, were prepared as described by Dupree and Sneed (1966). An otherwise identical diet containing no endrin served as the control.

Aquaria of 25-liter capacity supplied with 0.8 liter/min well water (28 ± 1 C) were stocked with 250 g of catfish (about 125 individuals). Each group was offered its respective diet at a rate of 1% of body wt/day for 198 days, after which all groups were offered an endrin-free diet for an additional 41 days. The individual groups were weighed after each interval in which 100 g of feed (dry weight) had been offered (about every 20 days), and a 20-g sample of fish was removed for residue analysis. The remaining fish were returned to the aquaria and the feeding level was adjusted to allow for the change in total weight.

To test the effect of endrin in water, we held 300 g of channel catfish (about 150 individuals) in 100-liter aluminum troughs supplied with 1.3 liters/min well water (28 ± 1 C). The fish were offered a commercial ration at a rate of 2% of body wt/day. Concentrations of 0.5 and 0.25 μg endrin/liter of water were maintained by metering an endrin–acetone solution in water (100 ml acetone/15 liters water) into the troughs. Fish samples of approximately 20 g were taken for analysis after 1, 2, and 5 days, and at weekly intervals thereafter for a total of 54 days. On day 55 the addition of endrin

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to the water was discontinued and samples were analyzed at weekly intervals for 28 days.

Fish samples were homogenized in a blender, mixed with anhydrous sodium sulfate, packed loosely in a chromatographic column, and eluted with 10% diethyl ether in hexane (Hesselberg and Johnson 1972). The eluate was partitioned with acetonitrile. Cleanup was effected by eluting with 5% diethyl ether in petroleum ether on a Florisil column.

Pesticide content of the samples was determined with a gas chromatograph equipped with an electron capture detector. A 1.8-m 2-mm I.D. glass column packed with 10% DC-200 on Gas Chrom Q was used for separation. Carrier gas flow was 40 ml/min and column temperature 200 C. Pesticide concentrations are based on wet weight, whole body analysis.

Effects of endrin in food — Endrin accumulated rapidly in the tissues of fish receiving 4.0–0.04 µg endrin/g of feed. The amounts of endrin in food, the mean (n = 3) concentration of endrin in the tissues on day 20 and from day 20 to day 198 (average of seven analyses during period, at about 3-week intervals; standard error in parentheses) were as follows:

Day of experiment	Endrin concentration in food (µg/g)				
	4.0	0.4	0.04	0.004	0.0004
20	0.31	0.037	0.006	ND	ND
20–198	0.307 (0.027)	0.05 (0.009)	0.008 (0.002)	ND	ND

Beginning on day 198, all groups were fed an endrin-free diet. Endrin was not detected 28 days later in fish receiving 0.4 and 0.04 µg endrin/g of feed. Residues in the group that received 4.0 µg endrin/g of feed had decreased to 0.011 µg/g in 28 days and were below detectable limits in 41 days.

Individual groups of fish receiving endrin in the diet showed no measurable correlation between endrin concentration and growth or mortality. Total mortality during the experiment was less than 1%.

Effects of endrin in water — Chronic exposure to endrin in water showed that rate of uptake and amount, as well as mortality, depended on the concentration in the water. Fish exposed to 0.5 µg endrin/liter of water had residues of 0.2 µg/g on day 5 and 0.38 µg/g by day 19. A hypersensitivity to sudden noise or movement was first noticed in this group on day 19 and was evident throughout the remainder of the experiment. Mortalities began on day 26, when endrin content of samples of nonmoribund fish used for analysis was 0.72 µg/g. On day 34, residue levels had increased to 1.0 µg/g and did not change appreciably by day 41, when all fish had died.

No mortality was observed in the group of fish exposed to 0.25 µg endrin/liter of water nor did the fish exhibit hypersensitivity. Residue levels were maximal on days 49 and 55 ($\bar{X}$ = 0.41 µg endrin/g). After addition of endrin to the water was stopped on day 55, tissue residues decreased to 0.02 µg/g within 13 days and to below detectable limits within 23 days.

Conclusions — The lack of high endrin concentrations in the tissues suggests a rapid turnover rate for the pesticide. This is further supported by the rapid decrease in concentration following withdrawal and indicates the pesticide could be readily cycled in closed aquatic systems (lakes, ponds, or intensive rearing conditions). What effect continual endrin cycling may have on the fish is not clear; however, continued exposure may result in increased tolerance. Ferguson et al. (1966) demonstrated that mosquitofish with a history of insecticide exposure were more resistant to endrin than those not having a history of exposure. The rapid decline in residue demonstrates that endrin could be eliminated in a relatively short time by closed system filtering or by holding the fish in continuous flow systems.

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Use of Plastein Reaction in Recovering Protein from Fish Waste

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ONOUE, Y., AND V. M. RIDDLE. 1973. Use of plastein reaction in recovering protein from fish waste. J. Fish. Res. Board Can. 30: 1745–1747.

A high quality proteinaceous product is recoverable in 35% yield from fish waste by a process of peptic hydrolysis followed by a pepsin-catalyzed recombination of soluble peptides to form plastein. Hydrolysis is at *p*H 2.0 and 37 C for 6 hr. Soluble peptides are then separated from insoluble residue, adjusted to *p*H 4.5, concentrated at 45 C to 30% protein solids, and incubated for 24 hr at 37 C with additional pepsin. The insoluble plastein formed during incubation is filtered, washed, and dried. In a pilot experiment with fillet waste from Petrale sole (*Eopsetta jordani*), the yield was 35% of total original protein. Proximate composition of the product was: protein, 96.1%; moisture, 2.9%; ash, 0.5%; and lipid, 0.0%. The plastein contained a higher proportion of essential amino acids than did a hydrolysate of the whole waste, all except tryptophan exceeding FAO reference standards.

ONOUE, Y., AND V. M. RIDDLE. 1973. Use of plastein reaction in recovering protein from fish waste. J. Fish. Res. Board Can. 30: 1745–1747.

Un produit du type protéique de haute qualité est récupéré avec un rendement de 35% des déchets de poisson par un procédé d'hydrolyse peptique suivie d'une recombinaison catalysée par la pepsine des peptides solubles pour former la plastéine. L'hydrolyse se fait à un *p*H de 2.0 et une température de 37 C pendant 6 h. Les peptides solubles sont ensuite séparés du résidu insoluble, ajustés à un *p*H de 4.5, concentrés à 45 C à 30% de solides protéiques et incubés pendant 24 h à 37 C, avec addition de pepsine. La plastéine insoluble formée durant l'incubation est filtrée, lavée et séchée. L'analyse par constituants donne: protéines 96.1%, humidité 2.9%, cendres 0.5% et lipides 0.0%. La plastéine contient une plus forte proportion d'acides aminés essentiels qu'un hydrolysat des déchets entiers, tous, sauf le tryptophanne, excédant les normes de références de la FAO.

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THIS is a report on a new approach to isolating fish protein of high purity and of high essential amino acid content from hydrolysates of fish wastes. Aqueous autolytic or enzyme-catalyzed hydrolytic processes to isolate protein appear to have certain advantages over organic solvent-based systems in terms of economy and simplicity of operation (Finch 1970). However, the primary products of such aqueous processes usually are of high ash content and contain bitter peptides necessitating sophisticated follow up procedures for their removal. In the study reported here, we utilize instead, the little-known "plastein" reaction (Fujimaki et al. 1971) as the follow-up step to isolate relatively pure protein. Proteolytic enzymes aid in partial hydrolysis of the waste protein and the same or different proteolytic enzymes subsequently catalyze the formation of higher molecular weight polypeptides (plastein) from smaller fragments. In the process, the plastein is separated from lipids, ash and other undesirable components of the hydrolysate and a high quality product is obtained.

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The technique — The procedure centers on use of proteolytic enzymes for hydrolysis of the waste protein and subsequently for catalyzing the formation of plastein from soluble peptides. Pepsin was the enzyme of choice in the current study because optimal activities for proteolysis and for plastein synthesis are in the *p*H range 2.0–4.35, preventing microbial activity.

Fish waste, with and without viscera, is comminuted in a meat grinder and homogenized with twice the weight of 0.24 N HCl, and the homogenates adjusted finally to *p*H 2.0. A portion of each homogenate, with 0.002% toluene added, is allowed to autolyze at 37 C. Periodically, 2.0-ml aliquots of the autolyzing solutions are withdrawn, weighed, combined with 1.0-ml portions of 12% trichloroacetic acid, and filtered. Total nitrogen content of the filtrates provides an approximate measure of the degree of hydrolysis of the protein.

Procedures identical to the preceding were carried out on two additional portions of the viscera-containing homogenate to which pepsin (Worthington Biochemical Corporation, crystallized, 3000 U/mg) had been added in ratios of enzyme to protein (E:P) of 1:400 and 1:140.

After 6 hr of incubation, all mixtures were adjusted to *p*H 4.5 with 2N NaOH and centrifuged at 8000 g for 10 min, and the supernatant fluids filtered through Whatman No. 1 paper. Using a Roto-Vac evaporator, the filtrates were concentrated to 30% protein solids

(*w*/*v*) at 45 C, then readjusted to *p*H 4.5. The concentrated filtrates from the autolyzed homogenates were then incubated at 37 C. Portions of the filtrates from the pepsin-treated homogenates were incubated similarly while other portions were first supplemented with additional pepsin (E:P, 1:200).

At the end of 24 hr of incubation, during which plastein formed and separated from solution, the mixtures were centrifuged, and the plastein washed with aqueous alcohol (50/50) and dried at room temperature under reduced pressure. The yield of plastein was calculated on the basis of total nitrogen in the original homogenate. Total nitrogen, moisture, lipid, and ash were determined by standardized procedures (AOAC 1965). Amino acid analyses were performed by a commercial laboratory.

Advantages of the technique — The technique, as described, was used in a pilot experiment on a laboratory scale with Petrale sole (*Eopsetta jordani*) fillet waste (Table 1). Under the test conditions, there was very little autolytic activity without viscera present. Hydrolysis was greatly accelerated by viscera, though plastein yield was increased only slightly, from 7 to 10%. Pepsin not only markedly increased the rate of hydrolysis but also had pronounced effects on plastein synthesis. The maximum yield of product, 35% of total original protein, was obtained with pepsin (E:P, 1:400) in the homogenate and supplemental pepsin (E:P, 1:200) present during plastein formation.

TABLE 1. Yield of plastein as influenced by pepsin in hydrolysates and in plastein synthesis.

	Hydrolysis (%)[c]		Yield of plastein (%)[d]
	0 hr	6 hr	
Viscera absent			
Pepsin not added	29	34	7
Viscera present[a]			
Pepsin not added	51	67	10
Pepsin added to homogenate only			
at 1:400[b]	52	81	15
at 1:140	55	100	13
Pepsin added to filtrate also, at 1:200			
1:400 in homogenate	52	81	35
1:140 in homogenate	55	100	22

[a]Viscera was ⅓ of the wet weight of the total fish waste.
[b]Enzyme:protein ratio.
[c]As measured by trichloroacetic acid precipitation.
[d]Percent of original protein recovered as plastein.

Excessive hydrolysis decreased the plastein yield as shown by the addition of pepsin (E:P, 1:140) to homogenates. Supplementation of this filtrate with pepsin (E:P, 1:200) enhanced the yield from 13 to only 22%.

Other advantages of using the plastein reaction in utilizing fish waste are illustrated by the quality of the product. The plastein obtained is odorless, tasteless, and only slightly off white in color. The proximate composition was protein, 96.1%; moisture, 2.9%; ash, 0.5%; and lipid, 0.0%. Also significant is the amino acid profile of the plastein (Table 2) compared with that reported for a different batch of sole fish waste homogenate (Tarky et al. 1973) and with the FAO amino acid reference pattern (FAO 1957).

One of the notable features of the plastein is the accumulation of disproportionate amounts of nonpolar essential amino acids, coupled with decreases in the proportions of polar nonessential amino acids. All essential amino acids in plastein, other than tryptophan, are in significant excess over those of the FAO reference pattern. That the product is insoluble may be no disadvantage when it is used as a nutritional supplement and may indeed prove to be a desirable attribute in some applications.

TABLE 2. Comparisons of amino acid profile of sole fish waste hydrolysate, plastein from sole fish waste, and 1957 FAO amino acid reference pattern.

	Grams/100 g protein		
Amino acid	Hydrolysate[a]	Plastein	Reference Pattern[b]
Lysine	8.7	7.8	4.2
Methionine	0.6	3.8	2.2
Total sulfur AA	–	4.5	4.2
Threonine	4.3	4.2	2.8
Tryptophan	–	1.1	1.4
Valine	4.7	7.4	4.2
Phenylalanine	4.0	6.3	2.8
Leucine	6.8	13.7	4.8
Isoleucine	3.9	9.3	4.2
Tyrosine	1.9	4.9	
Cysteine	–	0.7	
Aspartic	9.3	7.8	
Serine	5.3	3.3	
Glutamic	14.1	12.2	
Proline	5.9	3.0	
Glycine	12.3	2.8	
Alanine	7.7	3.7	
Histidine	1.1	2.2	
Arginine	8.0	5.6	

[a]Tarky et al. (1973).
[b]FAO 1957.

Discussion — The exact mechanism of plastein formation has not been established but is believed to involve coupling of peptide fragments via transpeptidation, with release of a terminal amino acid from one fragment. Such fragments must be tetrapeptides or larger and apparently require phenylalanine or tryosine carboxyterminal acids (Horowitz and Haurowitz 1959). When hydrolysis of protein is excessive, free amino acids and peptide fragments result which are too small to form plastein. The reaction is probably driven towards synthesis by the water-insoluble nature of the product as a consequence of the accumulation of nonpolar amino acids.

Acknowledgments — This research was supported in part by the National Oceanic and Atmospheric Office of Sea Grant under Grant Number NC-1-72.

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Some Aspects of Osmoregulation in *Mysis stenolepis* (Crustacea, Mysidacea)

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DORMAAR, K. A., AND S. COREY. 1973. Some aspects of osmoregulation in *Mysis stenolepis* (Crustacea, Mysidacea). J. Fish. Res. Board Can. 30: 1747–1749.

Osmotic regulation of the mysid *Mysis stenolepis* was studied by gradually and suddenly exposing specimens to salinities from 0 to 30‰. Acclimation to all salinities other than 0‰ occurred under all conditions within 3 hr. All specimens exposed to 0‰ died. *Mysis stenolepis* is hyposmotic (Δt 1.363 C) at 22–30‰ and hyperosmotic at salinities less than 22‰. The osmoregulatory pattern and the ability to acclimate rapidly indicates an adaptation to the estuarine environment.

DORMAAR, K. A., AND S. COREY. 1973. Some aspects of osmoregulation in *Mysis stenolepis* (Crustacea, Mysidacea). J. Fish. Res. Board Can. 30: 1747–1749.

Nous avons mesuré la régulation osmotique chez *Mysis stenolepis* en exposant des spécimens, graduellement ou soudainement, à des salinités variant de 0 à 30‰. L'acclimatation à toutes les salinités et sous toutes conditions s'est produite en moins de 3 h sauf à 0‰ où tous les spécimens exposés moururent. Dans l'intervalle de 22 à 30‰, *Mysis stenolepis* est hyposmotique (Δt 1.363 C), alors qu'il devient hyperosmotique à des salinités inférieures à 22‰. Les modalités osmorégulatrices et l'aptitude à s'acclimater rapidement témoignent de son adaptation à une vie en estuaire.

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Mysis stenolepis is a marine mysid inhabiting depths of littoral to 38 m along the eastern coast of North America from the Gulf of St. Lawrence to New Jersey (Smith 1879; Wigley 1964). Tattersal (1951) suggested that *M. stenolepis* was derived from *Mysis mixta*, moved inshore and has become adapted to living in shallow water.

Most truly marine Crustacea are polystenohaline, and show little if any osmoregulation, i.e. they are osmoconformers (Kinne 1963). On the basis of their osmoregulative capacity two groups can be distinguished, the hyperosmotic regulators and the hyper–hyposmotic regulators. The hyperosmotic regulators are hyperosmotic in higher salinities (Kinne 1963). The hyper–hyposmotic regulators are hyperosmotic in diluted seawater and hyposmotic in higher salinites viz. they regulated both at low and high salinities (Kinne 1963).

The factors controlling the distribution of *M. stenolepis* are virtually unknown. The osmoregulation of the haemolymph of *M. stenolepis* was studied to help determine the distributional pattern and the type of osmoregulatory pattern this species possesses.

Materials and methods — Specimens were collected in the summer of 1969 from shallow, open, intertidal waters along the east coast of New Brunswick (45°03′N; 67°03′W) by dragging a tow net at low speeds during the daytime near the bottom. Specimens were also collected from a nearby large intertidal pool.

Experimental animals were kept in two sets of seven 40-liter aquaria at 14 C (average summer temperature of the water), and at 75–79% O_2 saturation. Specimens from the two different localities were kept separately. The animals were held at a maximum density of two per liter. Food was provided by adding 500 ml of bottom mud to each tank. The water in each tank was slowly recirculated through a charcoal filter to maintain a low ammonia level (1.2 ppm). Dilutions of sea water starting at 30‰ were done at a rate of 5‰ over intervals of 4 days, until salinities of 25, 20, 15, 10, 5, and 0‰ were achieved. A control tank of 30‰ (sea water) was also maintained. In another set of tanks specimens were suddenly subjected to the different salinities all starting at 30‰. Salinities were determined daily with a hydrometer (range: 1.000–1.050) calibrated with standard sea water and deionized water at 4 C.

Daily haemolymph samples were taken from five specimens at each salinity and their freezing point depressions determined. The specimens were sampled once and then discarded. Specimens were first sampled 1 hr after exposure to the required salinity in each tank, and sampling continued until the freezing point depression remained constant. Only mature males and nongravid females, the stomachs of which contained food, were used.

To collect haemolymph, the dorsal carapace immediately posterior to the stomach was punctured with a fine pin. The tip (0.8 mm diameter) of a micropipette connected by a polyethylene tube to a modified RGI micrometer syringe was then inserted into the dorsal blood sinus. This entire system was filled with Cargilles "A" immersion oil. Approximately 2λ of haemolymph was taken by slowly turning the micrometer until enough haemolymph was collected and transferred to the platform of the freezing module of a cryostat osmometer (Clifton Technical Physics) for freezing point determination. The daily determinations of freezing point depressions at any of the six salinities were averaged. All the determinations of freezing point depressions of the seawater (30‰) specimens were averaged. To compare results at the different salinities and at sea water, 95% confidence levels were calculated for the daily determinations and for the salt water determinations from the two localities.

Results and discussion — The average freezing point depression of the haemolymph from *Mysis stenolepis* in 30‰ from the two localities were not significantly different (Δt 1.363 C and Δt 1.359 C, respectively). The freezing point depression of the haemolymph of animals gradually and suddenly exposed to salinities as low as 5% were constant within the first 3 hr of sampling. Animals acclimated to 5‰ and then gradually subjected to 0‰ died within 2 days when their internal freezing point decreased to Δt 0.653 C.

Mysis stenolepis was hyperosmotic from 5–21‰ and hyposmotic from 22–30‰ (Fig. 1). The freezing

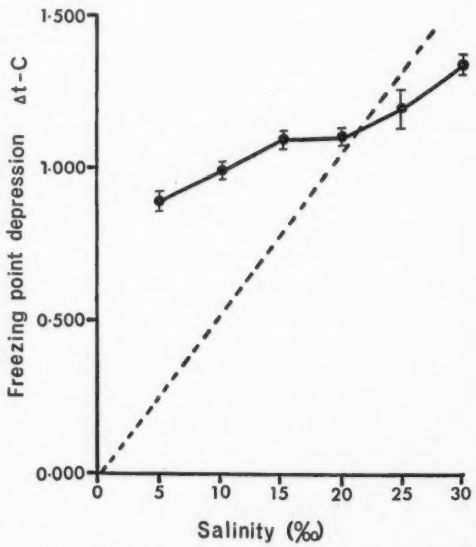

FIG. 1. Osmoregulatory pattern of *Mysis stenolepis* after acclimation to salinities of 5, 10, 15, 20, 25, and 30‰. Vertical bars represent absolute ranges. The broken line is the isosmotic line.

point depressions of the haemolymph ranged from Δt 0.653 to Δt 1.363 C.

After acclimation *M. stenolepis* showed a typical hyper–hyposmoregulatory pattern (Fig. 1), which is similar to certain other crustaceans *Uca crenulata*, *Neomysis integer*, *Palaemonetes varians*, and *Pachygrapsus crassipes* which are semiterrestrial or inhabit brackish waters the salinity of which fluctuates (Lockwood 1967). Kinne (1963) considers the hyper–hyposmoregulatory mechanism to be the most advanced stage of adaptation to fluctuating or extreme salinities. *Mysis stenolepis* acclimated to each salinity within 3 hr indicating a rapid ability to adjust to widely fluctuating salinities.

Mysis stenolepis has the osmoregulatory potential to inhabit areas of diluted low salinities or widely fluctuating salinities such as estuaries and certain coastal areas but is incapable of adjusting to salinities of 5‰ or lower and therefore would not invade freshwater habitats.

Acknowledgments — Facilities for carrying out part of this work were provided by the Fisheries Research Board of Canada, St. Andrews, N.B. Research was made possible through a National Research Council of Canada Grant to S. Corey.

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A Portable Wire–Speed Indicator for Use with Plankton Nets

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LUSZ, L. D., AND K. D. WALDRON. 1973. A portable wire-speed indicator for use with plankton nets. J. Fish. Res. Board Can. 30: 1749–1751.

A system is described for measuring true wire speed during plankton tows, using commercially available electrical components attached to a standard metering wheel. The indicator increases uniformity between and during plankton tows where the wire speed does not exceed 50 m/min.

LUSZ, L. D., AND K. D. WALDRON. 1973. A portable wire-speed indicator for use with plankton nets. J. Fish. Res. Board Can. 30: 1749–1751.

Nous décrivons un système permettant de déterminer la vitesse réelle du cable au cours de prises de plancton. Ce système utilise des composants électroniques accessibles sur le marché, fixés sur une poulie compteuse ordinaire. L'indicateur assure une plus grande uniformité entre les traits et durant les traits, pourvu que la vitesse du cable ne dépasse pas 50 m par minute.

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QUALITATIVE and quantitative variability between plankton collections may result from (1) distribution, abundance, and behavior of plankton organisms, and (2) methods used to obtain the collections. If methods used to capture plankton were completely uniform for all collections, all variability could be attributed to distribution, abundance, and behavior of the organisms. A part of the collecting procedure which contributes to uniformity between tows with plankton nets, especially oblique tows, is wire speed, i.e. the ability to maintain a predetermined rate at which the towing wire is paid out and retrieved.

Many winches used for towing plankton nets, especially those manufactured recently, are equipped with devices to measure wire speed. Some of these measure true wire speed while others approximate wire speed from the rate of rotation of the winch

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drum. Older winches and small portable winches are rarely equipped with such devices.

This note describes a system for measuring true wire speed during plankton tows, using commercially available electrical components attached to a standard metering wheel. Requirements were that it be (1) capable of continuous indication — to monitor setting and retrieval rates, (2) equipped with a remote readout — to allow the device to be used from different locations, (3) simple to

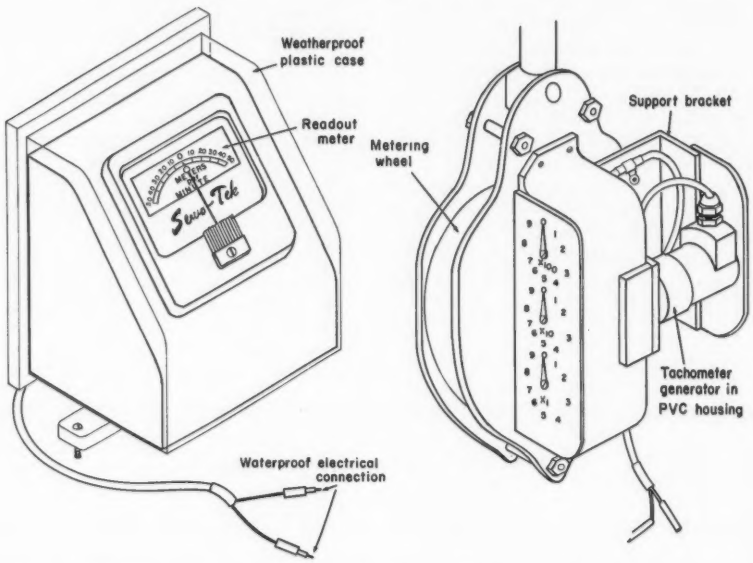

FIG. 1. Modified metering wheel and readout meter of a portable wire-speed indicator.

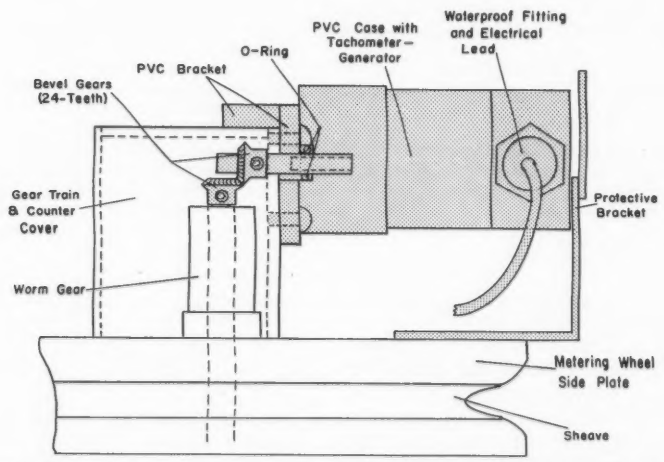

FIG. 2. Method used to couple tachometer–generator unit to metering wheel. Stippling indicates parts added to the original metering wheel.

operate — adaptable for routine use by crewmen from different vessels, (4) sturdily built — to withstand shipboard handling under a variety of sea conditions, and (5) self-contained — to eliminate external power leads and to increase portability.

Basic components of the device are a metering wheel, a tachometer–generator, and a readout meter (Fig. 1). The metering wheel was a half-meter circumference Atlas Model No. 1[1] with an integral dial type mechanical counter.

The tachometer-generator was a Servo-Tek Products Model ST-7253A-2[1] which provided an electrical output of 7 v d-c at 1000 rpm of the generator shaft. Although output was proportional to shaft speed, up to a maximum of 3600 rpm, actual operating speeds were in the range of 0–100 rpm. A pair of beveled gears with a 1:1 ratio was used to couple the tachometer–generator to the main shaft of the metering wheel (Fig. 2). The unit was mounted on the frame of the metering wheel in a weather-proof, polyvinyl chloride (PVC) housing. The electrical system was highly resistant to shock and vibration; brush and commutator design provided good stability with a long life expectancy under continuous operation.

The readout meter, obtained as part of the unit system, was a Servo-Tek Products Model ST-905 indicator calibrated so that 100 rpm provided a full-scale deflection from a center zero. Bi-directional rotation of the meter movement allowed monitoring of wire speed during both setting and retrieval of plankton nets. The readout meter was mounted in a weather-proof, clear plastic housing and connected to the tachometer–generator with 50 ft of 2-conductor insulated electrical cable. Weather-proof connectors were used to join the cable to the generator unit, and this permitted handling and installation of the metering wheel-generator unit separate from the readout unit. A longer cable could have been used, as accuracy of the system is relatively unaffected by the resistance of the connecting cable.

The readout meter was calibrated to indicate up to 100 rpm of the metering wheel, equivalent to 50 m/min. A hand-lettered dial face was prepared which permitted reading the meter in m/min rather than in rpm. The entire system had a capability of 100% overspeed without damage to the components, i.e. wire speeds of up to 100 m/min would be acceptable; however, there was no provision for indicating speeds greater than 50 m/min. Use of tachometer–generators with different outputs, and with appropriate readout meters, would permit measurement of different ranges of wire speeds.

Cost of the tachometer–generator and the readout meter, purchased as a unit system, was approximately $80 in 1971. The metering wheel was taken from our stock of equipment, and installation of the unit was done in our machine shop.

The wire-speed indicator described has been used successfully to monitor wire speed during about 100 plankton tows on two cruises of the RV *John N. Cobb* of the NMFS Northwest Fisheries Center, Seattle, Wash. Use of the device has allowed biologists to standardize plankton tows to a greater degree than was previously possible.

Acknowledgments—We wish to acknowledge the assistance of C. Wheeler and G. Esterberg of the Northwest Fisheries Center in modifying the metering wheel and in assembling components of the wire-speed indicator.

[1]Use of brand names does not imply endorsement of a particular product.

Occurrence of American Eels, *Anguilla rostrata*, in Waters Overlying the Eastern North American Continental Shelf[1]

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WENNER, C. A. 1973. Occurrence of American eels, *Anguilla rostrata*, in waters overlying the eastern North American Continental Shelf. J. Fish. Res. Board Can. 30: 1752–1755.

Eleven reproductively maturing specimens of the American eel, *Anguilla rostrata*, were collected during three independent off-shore trawling operations. Three females were taken on December 5, 1967 southeast of the mouth of Chesapeake Bay in 10–13 fathoms, one male and one female on November 5, 1969 southeast of Cape Cod in 35–45 fathoms, and six females east of Assateague Island on December 22, 1971 in 5 fathoms. Morphometrical analysis showed that the specimens were within the range of "silver" phase of *Anguilla rostrata*. Gonadal observations were made on all specimens.

WENNER, C. A. 1973. Occurrence of American eels, *Anguilla rostrata*, in waters overlying the eastern North American Continental Shelf. J. Fish. Res. Board Can. 30: 1752–1755.

Onze anguilles d'Amérique, *Anguilla rostrata*, en voie de maturation sexuelle ont été capturées aux cours de trois opérations indépendantes de chalutage dans les eaux du large. Trois femelles ont été capturées le 5 décembre 1967 au sud-est de l'entrée de la baie Chesapeake à une profondeur de 10 à 13 brasses, un mâle et une femelle le 5 novembre 1969 au sud-est du cap Cod par 35–45 brasses, et six femelles à l'est de l'île Assateague le 22 décembre 1971 par 5 brasses de profondeur. Une analyse morphométrique démontre que ces individus étaient tous dans les limites de la phase "argentée" d'*Anguilla rostrata*. Des observations ont été faites sur les gonades de tous les spécimens.

Received November 29, 1971

THE American eel (*Anguilla rostrata*) is a catadromous fish residing in estuaries and river systems from West Greenland (Jensen 1926), Labrador (Backus 1957), eastern Newfoundland, and the northern side of the Gulf of St. Lawrence south to the Gulf of Mexico, Panama, the West Indies to the northern coast of South America (Bigelow and Schroeder 1953). The complex life cycle has been summarized by Bigelow and Schroeder (1953), Vladykov (1955), Bertin (1956), and Eales (1968).

Although the annual fall migration of reproductively maturing specimens has been well documented, their capture in off-shore waters has not been reported. This communication reports the capture of three eels in 1967, two eels in 1969, and six eels in 1971 in waters overlying the continental shelf of the northeastern United States.

Materials and methods — The 1967 migratory eels were captured in an Atlantic Western trawl during a 1-hr tow by the *Sea Breeze*, a commercial trawler chartered by the Virginia Institute of Marine Science. The 1969 specimens were collected with a No. 36 Yankee trawl equipped with a ¾-inch stretch mesh cod end liner aboard the R/V *Albatross IV* during groundfish investigations of the U.S. Bureau of Commercial Fisheries. The 1971 specimens were collected during a 45-min tow with a 50-ft trawl equipped with a 1-inch stretch mesh cod end liner aboard the *Cynthia*, a commercial trawler chartered by the National Marine Fisheries Service Laboratory at Oxford, Md. The 1967 and 1969 bottom temperatures were taken from bathythermograph tracings while the 1971 bottom temperatures were taken by a stem thermometer; 1967 and 1971 bottom salinities were determined by an induction salinometer.

Vertebrae were counted from X-ray photographs, and morphometrics of the preserved specimens were taken using the method of Egd (1939). The 1971 specimens were frozen after capture. After thawing, the individual ovaries were removed and weighed to the nearest 0.1 g prior to formalin fixation. The maturity index (gónad weight/total weight) was calculated for these specimens. The longest horizontal diameter of 100 eggs from each ovary was measured at 150× magnification. Previous statistical analysis of ova diameters from specimens migrating from the Chesapeake Bay showed no significant differences between ovaries (Wenner 1972). Therefore,

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TABLE 1. Location and hydrographic data of off-shore specimens of *Anguilla rostraat*.

Specimen code	Date	Location		Depth (*fms*)	Temp. (*C*)		Salinity (‰)	
		Longitude	Latitude		Surface	Bottom	Surface	Bottom
A	Nov. 7 1969	41°40.5′N	69°39′W	45	10.8	8.5	–	–
B	Nov. 7 1969	41°38′N	69°42′W	30	10.8	9.0	–	–
C	Dec. 5 1967	35°51′N	75°30′W	10	10.7	10.7	31.66	30.94
D	Dec. 5 1967	35°41′N	75°18′W	12	11.3	11.4	31.58	31.81
E	Dec. 5 1967	35°25′N	75°18′W	13	12.0	12.0	31.14	32.21
F–K	Dec. 22 1971	37°51′N	75°19′W	5	8.0	8.0	28.43	30.97

TABLE 2. Morphometrics and vertebral counts of off-shore specimens of *Anguilla rostrata* along with morphometric ranges of silver eels migrating from the Chesapeake Bay, November 1970.

Specimen	A	B	C	D	E	F	G	H	I	J	K	Chesapeake Bay	
Sex	♀	♂	♀	♀	♀	♀	♀	♀	♀	♀	♀	♀	♂
Total vertebrae	108	109	108	108	107								
Total length (*mm*)	642	373	512	579	561	658	648	657	617	609	627		
Total weight (*g*)						525	568	543	540	529	529		
Preanal length (*mm*)	266	158	217	237	232	284	280	282	252	273	257		
Preanal length / Total length × 100%	41.4	42.3	42.4	40.9	41.3	43.2	43.2	42.9	40.8	44.8	41.0	36.6–45.2	39.5–43.8
Preanal-head length (*mm*)	188	106	147	159	161	197	194	195	168	196	183		
Preanal-head / Total length × 100%	29.3	28.4	28.7	27.5	28.7	29.9	29.9	29.7	27.2	32.2	29.2	25.6–32.2	25.4–30.9
Specimen	A	B	C	D	E	F	G	H	I	J	K	Chesapeake Bay	
												♀	♂
Predorsal length (*mm*)	*221*	*126*	*168*	*195*	*187*	*220*	*223*	*218*	*203*	*217*	*212*		
Predorsal length / Total length × 100%	34.3	33.8	32.8	33.7	33.3	33.4	34.4	33.2	32.9	35.6	33.8	30.8–36.7	29.1–35.6
Predorsal-head length (*mm*)	143	74	119	117	116	133	137	131	119	140	138		
Predorsal-head / Total length × 100%	22.3	19.8	23.2	20.2	20.7	20.2	21.1	19.9	19.3	23.0	22.0	17.5–24.6	16.0–24.1
Preanal-predorsal length (*mm*)	45	32	49	42	45	64	57	64	49	56	45		
Preanal-predorsal / Total length × 100%	7.0	8.6	9.6	7.2	8.0	9.7	8.8	9.7	7.9	9.1	7.2	4.5–11.5	5.4–11.8
Specimen	A	B	C	D	E	F	G	H	I	J	K	Chesapeake Bay	
												♀	♂
Head length (*mm*)	78	52	70	78	71	87	86	87	84	77	74		
Head length / Total length × 100%	12.1	13.9	13.7	13.5	12.6	13.2	13.3	13.2	13.6	12.6	11.8	10.5–13.8	11.2–14.1
Gape length (*mm*)	17.2	11.3	17.9	21.8	17.4	21.5	24.5	18.5	18.8	18.1	19.4		
Gape length / Head length × 100%	22.0	21.7	25.6	27.9	24.5	24.7	28.5	21.2	22.4	23.5	26.2	19.7–32.1	16.7–26.9
Horizontal diameter of the eye (*mm*)	*8.5*	*6.5*	*8.0*	*9.6*	*7.9*	*10.2*	*10.0*	*9.7*	*11.1*	*8.8*	*9.0*		
Diameter / Gape length × 100%	49.4	57.5	44.7	44.0	45.4	47.4	40.8	52.4	59.0	48.6	46.4	34.2–68.8	42.0–100.0
Specimen	A	B	C	D	E	F	G	H	I	J	K	Chesapeake Bay	
												♀	♂
Left ovarian weight (*g*)						18.5	14.9	16.9	18.9	13.8	10.9		
Right ovarian weight (*g*)						18.4	14.9	16.8	17.1	16.4	12.7		
Total ovarian weight (*g*)						36.9	29.8	33.7	36.0	30.2	23.6		
Maturity index						.0702	.0524	.0620	.0660	.0570	.0446		

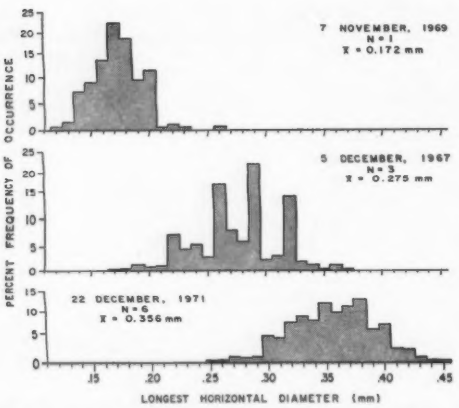

FIG. 1. Frequency distributions of ova diameters from off-shore American eels (*Anguilla rostrata*).

the ova diameters from both ovaries of individual fish were pooled and tested for significant differences between fish by analysis of variance (Guenther 1964).

Results and discussion — Hydrographic data and sites of capture are found in Table 1; morphometrics and vertebral counts are in Table 2. Frequency distributions of ova from the three collection dates are found in Fig. 1.

The conclusion that all of the off-shore specimens were silver eels was based on external morphology and coloration and gonadal condition. The horizontal diameter of the eye expressed as percent of the gape length of all fish ($\bar{x}$ = 48.7%; range, 44.0–59.0) was statistically greater (F = 13.973; df = 1165) than values obtained for 156 female yellow eels ($\bar{x}$ = 41.8%; range, 30.7–59.1) collected from Chesapeake Bay sub-estuaries (C. A. Wenner unpublished data). The dorsal aspect of the pectoral fins and the caudal fin were darkly pigmented and the lateral line was prominent in all off-shore specimens. The general body coloration was that of a "silver" eel, or as Vladykov (1955) described migratory American eels, "bronze" eels. The gross appearance of the gonads of all specimens showed a stage of maturity that resembled the gonadal state of eels migrating from the Chesapeake Bay.

An analysis of variance showed a highly significant difference (F = 969.60, df = 9, 1990) in ova diameters between fish. All morphometric values fell within the ranges of values for silver *A. rostrata* leaving Chesapeake Bay during November 1970 (see Table 2). The ova diameters of the 1967 fish were also within the ranges of ova diameters of fish migrating from the bay ($\bar{x}$ = 0.27 with a range from 0.17–0.37 mm). The date of capture closely coincides with the peak of the eel fishery in lower Chesapeake Bay, but the possibility of a different estuarine origin cannot be excluded.

Gray and Andrews (1970) reported ova diameters ranging from 0.109 to 0.214 mm with a mean of 0.165 mm in silver eels leaving Newfoundland waters. These values are close to those of the 1969 female silver eel collected southeast of Cape Cod ($\bar{x}$ = 0.17, range, 0.12–0.27 mm). The 1971 eels had larger ova and greater maturity indices than any other specimens encountered in this study. The gonads comprised a mean of 5.78% of the total weight and the ova had a mean diameter of 0.356 with a range from 0.25 to 0.45 mm.

Although the 1971 specimens are more sexually mature as judged by ova diameters, their estuarine origin and the gonadal condition at the time of entrance into the sea is uncertain. Vladykov (1955) states "the exact routes of the eels in the sea and their behavior are not yet known." The possibility that migratory eels follow the coastline down to some region and then cross the Gulf Stream to reach the spawning grounds cannot be ruled out. It appears that eels from Chesapeake Bay have larger eggs when they enter the sea and migrate later in the season than eels of more northerly estuaries. Consequently, fish from different estuaries may reach the spawning area at approximately the same time and the same reproductive state.

Acknowledgments — I thank Dr M. Grosslein of the National Marine Fisheries Service for providing the 1969 specimens and Dr J. A. Musick and Mr J. McEachran of the Virginia Institute of Marine Science for the 1967 specimens and their critical reading of the manuscript. I also thank Dr F. O. Perkins of the Virginia Institute of Marine Science for his concern and consideration.

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An Estimate of Energy Expenditure by Rainbow Trout (*Salmo gairdneri*) in a Small Mountain Stream

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FELDMETH, C. R., AND T. M. JENKINS JR. 1973. An estimate of energy expenditure by rainbow trout (*Salmo gairdneri*) in a small mountain stream. J. Fish. Res. Board Can. 30: 1755–1759.

A method is presented which estimates energy expended by rainbow trout (*Salmo gairdneri*) in a natural stream habitat. Swimming speeds were determined by counting caudal fin beat frequency in the field. Published data on metabolic rates estimated in a water tunnel respirometer were then used to calculate energy expenditure.

Swimming speeds and hence energy expenditure did not vary statistically for time of day or night (overall means: swimming speed, 16.1 cm/sec; energy expenditure, 736.5 cal/kg per hour). Our calculations indicate that swimming at 16 cm/sec would cost a 100-g rainbow trout 53 cal/hr at 15 C, and about 0.48 g (live weight) of natural food per 6 hr would be needed to offset the cost of swimming.

FELDMETH, C. R., AND T. M. JENKINS JR. 1973. An estimate of energy expenditure by rainbow trout (*Salmo gairdneri*) in a small mountain stream. J. Fish. Res. Board Can. 30: 1755–1759.

Nous décrivons une méthode pour estimer l'énergie dépensée par des poissons d'eau douce nageant dans un cours d'eau naturel. Nous avons déterminé la fréquence des battements de la caudale de la truite arc-en-ciel (*Salmo gairdneri*) dans le cours d'eau. En utilisant la relation entre cette fréquence et la vitesse de nage mesurée dans un respiratomètre d'une vitesse d'écoulement d'eau variée, on a pu calculer la vitesse de nage du poisson. Nous avons aussi estimé le métabolisme en utilisant la relation entre cette vitesse et la consommation d'oxygène. Celle-ci a été convertie en calories par le biais de l'équivalent calorifique.

La vitesse de nage des truites, et partant la dépense d'énergie, ne varie pas de façon appréciable au cours de la journée ou de la nuit (vitesse moyenne: 16.1 cm/sec; dépense d'énergie moyenne 736.5 cal/kg par heure). Les calculs indiquent qu'une truite arc-en-ciel de 100 g dépense 53 cal/h à 15 C ou 318 cal/6 h en nageant à une vitesse de 16 cm/sec. Cette truite doit manger 0.48 g par 16 h de sa nourriture naturelle pour obtenir assez d'énergie pour nager.

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MEASURING energy output of undisturbed fish in natural environments has been handicapped by one difficulty in particular. It has been shown that fish elevate their metabolic rates for as long as 8–12 hr after handling (Graham 1949; Beamish and Mookherjii 1964; Kutty 1968; Rao 1968). Hence, any methods which require manipulation of fish prior to measure of energy output are probably overestimates. Energetics of undisturbed fish have been studied in laboratory streams (Averett 1969; Brocksen et al. 1968), but swimming and feeding behaviour under such circumstances may not be typical of fish in natural habitats; moreover, the method is time-consuming and requires sophisticated apparatus.

The object of our study was to estimate the energy expended day and night by undisturbed fish swimming in a stream. We approached the problem indirectly by determining swimming speeds of fish in their normal habitat and relating them to laboratory swimming speed–oxygen consumption data. Since stream flow is often turbulent and fish are undoubtedly exposed to constant changes in currents, we were unable to obtain consistent direct measurements of the current velocity fish occupied in a stream. However, we believe we were able to establish a valid relationship between caudal fin beat frequency and swimming speed, from which we could estimate metabolic rate and energy expenditure in the manner described in this paper.

Materials and methods — Fin beat vs. swimming speed — Rainbow trout (*Salmo gairdneri*) ranging in weight from 72 to 356 g, and in length from 16.7 to 27.6 cm (standard length), were used in this study. The swimming speed-caudal fin beat relationship was determined by placing fish in a Blazka-type water-tunnel respirometer similar to that described by Smit (1965). After a 12–18-hr adjustment period, during which fish swam at 15–20 cm/sec, caudal fin beats were observed over a series of current speeds (15.5–51.7 cm/sec). Stream water ranging in temperature from 11.0 to 13.5 C was constantly circulated through the water tunnel. The caudal fin beat frequency was observed for each fish 10 successive times by measuring the time required for 100 tail beats. The current velocity was then set at a new speed and counting began 30 min later.

Metabolic rate vs. swimming speed — Metabolic data for swimming rainbow trout in a similar water-tunnel respirometer have been accumulated by Rao (1968) for a large number of fish swimming at a variety of current speeds at temperatures of 5 and 15 C. Occasional measurements of oxygen consumption of swimming trout in our water tunnel compared closely with Rao's data for a given temperature and swimming speed. Hence, a relationship of oxygen consumption to swimming speed was taken from Rao's data for fish of 100 and 300 g swimming in temperatures of 5 and 15 C (Fig. 1).

Fin beat frequency in field — We placed rainbow trout into a small, controlled-flow stream (discharge, 0.03 m:/sec) diverted from Convict Creek in the Sierra Nevada of eastern California. Two fish were retained in each of three separate stream sections in a manner previously described by Jenkins (1969). After a 2–3 day adjustment period, observations of caudal fin beat frequency were made from towers concealing the observer. Fin beat frequencies and general behavior were observed over 6-hr periods during the day or night, and fin beats were recorded hourly after the first hour. Each fish was tagged with a white cellulose-acetate disc or triangle just posterior to and below the dorsal fin, to aid in counting fin beats at night. During the dark periods, observations were made with an electronic light intensifier (magnification approx 32,000X). With this instrument we could discern tail beats without artificial illumination even under starlight.

Calculation of energy expenditure — After the 6-hr observation period, fish were removed from the stream and their lengths and weights were measured. Metabolic energy expenditure, expressed as oxygen consumption per kilogram of fish weight per hour, was then estimated by determining swimming speed from the caudal beat

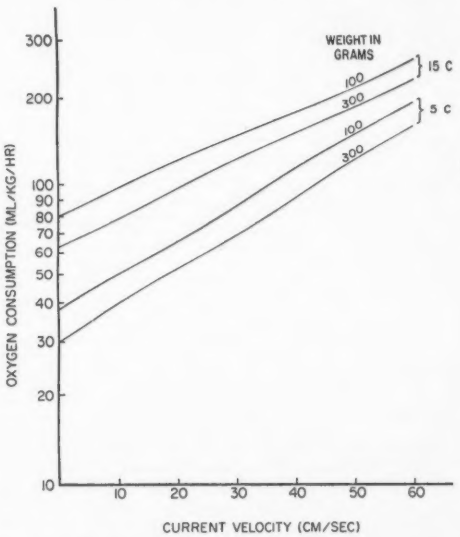

FIG. 1. Relationship between swimming speed and metabolic rate expressed as milliliters oxygen consumed per kilogram per hour. Values of oxygen consumption for 100-g and 300-g rainbow trout (*Salmo gairdneri*) in 5 and 15 C were plotted from regression equations determined by Rao (1968).

frequency-swimming speed relationship (Fig. 2), and determining the rate of oxygen consumption at the mean swimming speed from the metabolism-swimming speed curve (Fig. 1).

To convert respiration rate to calories of energy expended, a caloric equivalent for oxygen had to be determined. Since the amount of oxygen involved in fuel consumption depends on the proportions of different fuels utilized, some assumptions had to be made concerning the nutritional state of Rao's rainbow trout. At the time Rao made oxygen consumption measurements, his fish had been deprived of their commercial trout food for at least 36 hr. We can suppose that gastric evacuation had just terminated in his 15-C group and was probably in its later stages in 5-C groups (Windell et al. 1969), so the fish were more likely to be utilizing recently acquired fuels than stored fuels (e.g. liver glycogen and fats). Assuming 1) a common hatchery diet containing energy foods in the proportions 90% protein to 10% lipids (Phillips et al. 1966); 2) 65% extractable energy from protein and 85% from fat (Phillips and Brockway 1959); and 3) caloric equivalents for oxygen of 4.716 and 4.486 while metabolizing fats and proteins, respectively, we derive a combined caloric equivalent for oxygen of 4.516 kcal/liter. This figure suggests that oxygen consumption would be unusually high at a given level of energy expenditure compared to that of a fasting fish metabolizing liver glycogen or stored fat (5.007 and 4.686 kcal/liter oxygen, respectively), Since metabolism was reported to have dropped to fasting levels at the time of experiments, we favor instead the assumption of pure carbohydrate metabolism at 5.007 kcal/liter. This assumption is consistent with measured R.Q.'s of about 1.0 for rainbow trout (Kutty 1968).

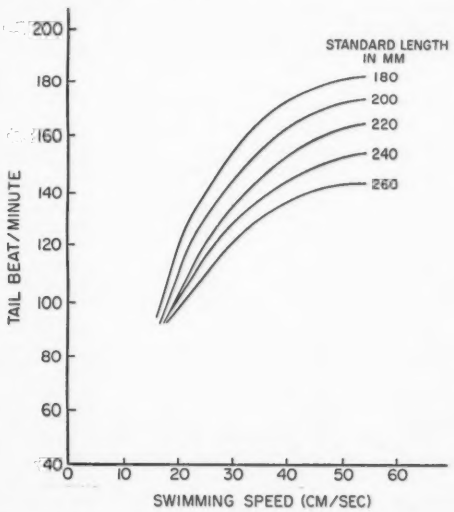

FIG. 2. Relation between swimming speed and tail beat frequency determined in a Blazka-type water tunnel-respirometer (Smit 1965). Lines are drawn through mean values of 10 observations at swimming speeds of 15.5, 24.5, 33.8, and 51.7 cm/sec.

Results and discussion — Rainbow trout in our stream swam constantly day and night in characteristic holding positions. Their mean swimming speeds ranged from 15.8 cm/sec during the morning observations period (0700–1300) on September 21 to 18.0 cm/sec during the afternoon period (1200–1800) on October 12 (Table 1). Differences between fish in swimming speeds were not significant statisically ($P > .05$).

Mean energy expenditure by the trout for the time periods studied ranged from 561.2 to 843.4 cal/kg/per hr. Because energy expenditure depended directly on swimming speed, and water temperature varied little (11.0–13.5 C), fish of similar size showed only slight differences in energy expenditure for the four time periods studied (Table 1). Hence rainbow trout in the small mountain stream we studied expended energy at a nearly uniform rate throughout the day and night.

The method of energy expenditure calculation presented in this paper allows instantaneous estimates of caloric output to be made in field situations. Data presented for rainbow trout probably could be used for other stream-dwelling salmonids within the size and temperature range for which we present data. If one can account for the expected small differences between trout species in standard metabolism, a good estimate of the caloric cost increment due to swimming at a given velocity and temperature can be made using this method.

The current velocity of a particular trout location in a stream cannot in our experience be measured accurately, but fin beat frequency averaged over a period of time should give a good estimate of swimming speed and hence energy expenditure. If trout were to increase swimming speed by increasing beat amplitude rather than frequency, our conclusions might be questioned. However, Hunter and Zweifel (1971) concluded that speed and frequency are consistently related, and their equations for the fin beat–speed relationship in rainbow trout (derived from Bainbridge 1958) give results similar to our empirical measurements.

Since fish length greatly affects the tail beat–swimming speed relationship (Bainbridge 1958) it is essential that an accurate fish length measurement be made. In our stream we were able to remove fish after a 6-hr swimming speed observation period by using two large dipnets. In other field situations, fly fishing, rifle or shotgun fire, or electrofishing might be suitable methods of fish capture after tail beat counts have been made.

Swimming speed rates for the four time periods studied were remarkably similar (Table 1). Rainbow trout are definitely known to feed at night (Jenkins 1969b), and the observations in this study indicate that they hold stream positions which require constant swimming activity. According to our calculations, swimming at 16 cm/sec would cost a 100-g fish 53 cal of energy per hour at 15 C. The amount of natural food required to offset this expenditure can be calculated in the following manner.

In 6 hr, the 100-g rainbow trout would use about 318 cal for swimming and maintenance. As noted in the methods section, trout can extract about 69% of the energy from proteins and 85% from fats. Using an average composition (ash free dry weight) of natural Convict Creek foods (84% protein, 16% fat, Phillips et al. 1954) we find a composite physiological value for natural food of 72%. With this figure and the mean caloric equivalent for ash free dry weight of eight stream insect species (5700 cal/g, Cummins and Wuycheck 1971) we find that these insects yield to trout 4104 cal/g consumed. Therefore a 100-g trout would require about 0.077 g ash free dry weight of food to offset its minimum energy needs in a stream for 6 hr. The same stream foods are about 82% water and 2% "ash," so in terms of live organisms the equivalent would be about 0.48 g/6 hr or 1.92 g/day.

When feeding was measured for rainbow trout in our observation stream (Feldmeth and Jenkins unpublished data), only one fish of the eight which had measurable stomach contents captured more than this necessary "stream maintenance ration" during a 6-hr period.[1] Although we feel that poor feeding by some of the fish was due to stress not

[1]Fish were stream acclimated for 2–3 days, net captured at the beginning of a 6-hr observation period, anesthetized in M.S. 222, and their stomachs were emptied with a small brass double tube pump. The entire procedure took 1–2 min, and the fish assumed normal swimming and agonistic activity (although evidently not always normal feeding activity) immediately when replaced in the stream. Six-hour food consumption was determined by repumping or sacrifice at termination of the experiment.

TABLE 1. Time periods, water temperature, standard length, weight, mean swimming speed, oxygen consumptions, and energy expenditure in calories per kilogram per hour for rainbow trout (*Salmo gairdneri*). Means and 95% confidence intervals for six trials are presented. Mean swimming speed and mean energy expenditure were not significantly different for the four time periods.

Date	Time period (*PST*)	Water temp. (C)	Standard length (*cm*)	Weight (*g*)	Mean swimming (*cm/sec*)	O_2 consumption (*ml/kg/hr*)	Cal/kg/hr
Sept. 21	0700–1300	13.5	24.48	279.8	15.83	112.0	561.2
			1.71	68.5	4.62	38.2	383.7
Sept. 30	1300–1900	11.0	22.48	226.7	15.58	131.2	657.2
			3.55	115.7	2.13	48.2	243.0
Oct. 1[a]	1900–0100	11.5	19.43	119.3	15.43	156.7	666.2
			1.30	31.3	0.95	13.4	451.5
Oct. 9[a]	0100–0700	12.0	19.3	123.7	16.02	168.7	843.4
			2.30	53.1	2.03	25.2	433.8
Oct. 12	1200–1800	12.0	19.88	136.3	18.1	159.7	800.0
			1.47	32.1	3.79	27.9	177.9
Oct. 18	1200–1800	11.5	20.48	143.8	15.9	155.2	777.6
			2.92	22.9	4.4	18.2	96.9
				overall mean	= 16.1		736.5

[a]Night illumination at 2000 PST (full moon) 1.0×10^{-3} to 1.4×10^{-2} ft-c. At 2400 PST (moon overhead) 2.5×10^{-2} ft-c.
[b]Night illumination at 2325 PST (half moon overhead) 4.7×10^{-3} ft-c.

manifested in general behavior, even wild Convict Creek fish seem rarely to encounter a wealth of food. As they must acquire additional energy for social behavior, predator avoidance, and reproduction, and for sparing materials for gonadal and somatic growth and maintenance, it is not surprising that few wild fish in Convict Creek exceed the size of our smaller experimental fish.

Acknowledgments — We thank the California Department of Fish and Game for providing the fish and Mr G. V. Elliott for assistance in making swimming speed observations in the field. We also wish to thank the U.S. Army for the loan of the electronic light intensifier.

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Inability to Detect Spores of *Clostridium botulinum* in Fish Protein Concentrates (FPC)

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Samples of fish protein concentrates from a total of 124 production lots prepared in pilot plants by three different methods have been tested for the presence of viable *Clostridium botulinum* spores by incubating them in cooked meat medium and assaying the culture supernatant fluids for toxin. No spores of *C. botulinum* could be demonstrated with certainty in any of the 124 samples of FPC tested. Two of the supernatant fluids were lethal to mice, but their lethal effects appeared to be caused by factors other than *C. botulinum* toxin.

HAUSCHILD, A. H. W., H. PIVNICK, AND L. W. REGIER. 1973. Inability to detect spores of *Clostridium botulinum* in fish protein concentrates (FPC). J. Fish. Res. Board Can. 30: 1760–1762.

On a préparé en ateliers pilotes, selon trois méthodes différentes, des concentrés de protéines de poisson, dont 124 lots furent analysés en vue de déceler la présence de spores viables de *Clostridium botulinum*. Les échantillons furent incubés en milieu de viande cuite, et la présence de produits toxiques dans les surnageants vérifiée par analyse biologique. On n'a pas détecté avec certitude de spores de *C. botulinum* dans les 124 échantillons de FPC analysés. Deux de ces liquides se sont avérés fatals aux souris, mais, de toute évidence, la mort fut causée par des facteurs autres que la toxine de *C. botulinum*.

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THE large-scale production of fish protein concentrates (FPC) for human consumption raises concern about the level of contamination with *Clostridium botulinum* spores, particularly of type E which is the most frequent isolate from fish (Bott et al. 1966; Cann et al. 1966; Craig et al. 1968). In this study, no spores of *C. botulinum* could be demonstrated with certainty in any of 124 samples of FPC tested.

Materials and methods — The samples were divided into three groups. The lots of groups A and B were prepared in the pilot plant of the Halifax Laboratory, Fisheries Research Board of Canada. The raw materials were obtained from commercial sources, and standard manufacturing practices were used to limit microbial contamination. The 38 lots of group A were prepared by the hot isopropanol extraction method of Power (1962) from skate, dogfish, cod frames and fillets, flounder, ocean perch, sand lance, swordfish, herring, capelin, and haddock frames. The 62 lots of group B were prepared from cod fillets by a more gentle procedure (unpublished, patent pending) which includes dispersion of the meat in water, centrifuging, resuspension of the sediment in dilute brine, *p*H adjustment to 8.5, holding, adjusting to *p*H 4.5, heating and cooling, adjusting to *p*H 6.9, centrifuging, three repeated lipid extractions of the sediment with isopropanol at ambient temperature, drying in a rotating cone vacuum drier at 70–75 C, and grinding in a Hammer mill. The 24 lots of group C were prepared by hot isopropanol extraction (Ernst 1971; Knobl et al. 1971) of hake, menhaden, and anchovy at the Experiment and Demonstration Plant, National Marine Fisheries Service, Aberdeen, Wash. All shipments were made in sterile polyethylene bags, each containing about 50–100 g of FPC.

Duplicate aliquots of 1.0–1.5 g from each FPC sample were added to 15 ml of cooked meat medium (Difco) in 20- × 150-mm screw-cap test tubes. The tube contents were stirred until the protein was completely wetted. For spore activation and destruction of the vegetative flora, one set of tubes was heated at 80 C for 10 min. Because of the heat sensitivity of *C. botulinum* type E spores, the duplicate set was heated at 60 C for 20 min.

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The contents were covered with Vaspar (Pivnick and Thacker 1970), and the tubes were incubated at 30 C for 4 days and subsequently at 22–24 C for 3 days. The fluid contents of all tubes with signs of growth were harvested, pooled with the fluid contents of the corresponding duplicate tube, and centrifuged at 11,000 rcf for 10 min. The supernatant fluids were injected intraperitoneally (ip) in volumes of 0.25 ml into white mice (20–22 g) using one mouse per sample. For trypsin activation of the type E toxin, the method of Duff et al. (1956) was slightly modified by mixing supernatant fluids 1:1 with 0.1 M potassium phosphate, *p*H 7.0, containing 2% trypsin (1:250; Difco) and incubating the mixtures at 37 C for 30 min. The mixtures were cooled and injected ip in volumes of 0.5 ml into mice. Deaths were recorded for 4 days after injection. The storage temperature for the supernatant fluids was 4 C.

Whenever a mouse died after injection with untreated or trypsin-treated supernatant fluid, the injections were repeated in triplicate. If deaths were recorded again, the animals were first injected with 0.5 ml of either polyvalent (types A–F, Statens Serum Institute, Copenhagen, Denmark) or monovalent (type E, Connaught Medical Research Laboratories, Willowdale, Ont.) *C. botulinum* antitoxin and injected 30 min later with the supernatant fluids as described above.

Results and discussion — Of the 124 FPC samples, 112 gave rise to growth in at least one of the duplicate tubes, usually in both. In most tubes, growth was accompanied by considerable gas formation. All control tubes (without added FPC) remained clear. Of the 12 FPC lots that did not initiate growth after heat treatment at either 60 or 80 C, eight belonged to sample group A, one to group B, and three to group C.

No deaths were caused by any of the 38 samples of group A, with or without trypsin activation. Deaths were recorded in mice injected with untreated samples N2, N3, and N49 (group B) and with trypsin-treated samples N14, N18, N21, N22 (group B), and B0510-1405 (group C). With two exceptions (N14 and B0510-1405), repeat injections of these samples in triplicate caused no more deaths. Without trypsin treatment, these two samples caused deaths in 2/6 and 0/3 mice, respectively; with trypsin treatment, they caused deaths in 3/9 and 1/3 mice, respectively, (Table 1, Exp. 1).

For these two samples, the test was repeated without mixing the cultures originally heated at 80 and 60 C (Table 1, Exp. 2). Five out of a total of 12 mice injected with supernatant fluid N14 died, while no further deaths were recorded after injection of supernatant fluid B0510-1405. Although the animals injected with sample N14 did not show the symptoms characteristic of botulism in mice, i.e. heavy breathing and unbalanced movement, a neutralization test was performed (Table 1, Exp. 3). In each group of mice protected with antiserum, 1/6 mice died. It is unlikely, therefore, that death was caused by *C. botulinum* toxin.

Although we were unable to demonstrate the presence of *C. botulinum* spores, it is likely that the incubation of larger FPC samples in larger volumes of cooked meat broth would have led to detection of such spores. However, the results indicate that any contamination of the FPC samples with *C. botulinum* spores would be moderate. Nevertheless,

TABLE 1. Toxicity of supernatant fluids of cultures initiated with two FPC samples.

Exp.	FPC lot	Activation temp. (C)	Trypsin treatment	Antiserum treatment	Mice killed per total no. inj.
1	N14	60+80[a]	–[b]	–	2/6
			+	–	3/9
	B0510-1405	60+80	–	–	0/3
			+	–	1/3
2	N14	60	–	–	1/3
			+	–	1/3
		80	–	–	0/3
			+	–	3/3
	B0510–1405	60	–	–	0/3
			+	–	0/3
		80	–	–	0/3
			+	–	0/3
3	N14	80	+	–	3/8
			+	A–F	1/6
			+	E	1/6

[a]Combined.
[b]+, Addition made; – No addition made.

it should be emphasized that all the FPC samples tested here were derived from pilot plants.

From the number of tubes in which growth was initiated by FPC samples, it appears that contamination with nonbotulinal spores was larger in FPC prepared by method B (and possibly method C) than in FPC prepared by method A. This difference may be related to the relatively mild heat treatment provided in method B.

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Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal Fisheries Research Board Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries*. L'auteur doit fournir un *résumé*. Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être exécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscript, il doit spècifier que son institution accepte les frais.

Liste de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).

Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral *one*, and between the capital letter O and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal
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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des tableaux numérotés.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: *Laissez trois espaces au-dessus et au-dessous des lignes* renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Canadian Nature Federation

An Open Letter to Bioscientists in Canada

This letter is being sent to all the biologists in Canada whom we can identify. It is a warm invitation to join the Canadian Nature Federation and to receive the four numbers of our magazine, *Nature Canada*, which go to members each year. We believe that you will recognize that there are many values of membership to you and to others in your family.

We, Ted Mosquin and Doug Pimlott, are biologists, one on the botanical and the other on the zoological side of things. We have had a long-term interest in natural history and for many years have been members of naturalists' organizations in Canada. Over the course of years we have developed deep convictions about the value of these organizations to Canada. We see naturalists' organizations as playing strong roles in portraying the natural environments of the country to people who have not tended to think much of nature as part of the cultural heritage of our country. In addition, we believe that these organizations constitute a vital link between people and governments in matters concerning the problem of the Canadian environment.

Because of these convictions we have worked hard during the past three years to bring about the evolution of the CNF as a strong national organization. We have not done it alone of course; it required the cooperation of many people and organizations.

Now CNF is away and running. Our membership grew from 1800 to 5000 in 1972 and is over 8000 at present. Six issues of *Nature Canada* have been published and the magazine has been widely acclaimed. In addition to portraying nature to Canadians in an impressive way, it has brought important environmental questions to the attention of Canadians. We consider, for example, that the development of water resources and protection of the North are extremely important to the future of Canada so these issues have been dealt with in a particularly incisive way in *Nature Canada*. Through special publications and briefs, the Federation has become more directly involved in environmental issues.

To sum up, we decided to write this letter because we believe that it is vital to the role of the Canadian Nature Federation that its membership consist of a strong blend of Canadians from all walks of life; from those who have traditionally supported organizations such as ours, to people who appreciate nature in less active ways, to professionals who can provide advice and expertise on complex environmental questions. Our personal experience convinces us that the arrangement will not be one-sided.

We urge you to join for a year and judge for yourself.

DOUGLAS H. PIMLOTT
President

THEODORE MOSQUIN
Executive Director

The Lobster (Homaridae) and the Lobster Fisheries:

An Interdisciplinary Bibliography

Compiled by W. S. W. Nowak

The book is divided into 2 sections:

Section 1

a) Scientific works comprised in scientific journals
b) Works in non-technical journals written by scientists
c) Works by non-scientists in scientific journals

Section 2

a) Geographical Index
b) Taxonomic Index
c) List of authors and experts cited
d) Subject Index

Marine Sciences Research Laboratory Technical Reports No. 6 (1972)

Price: $2.00 *Pages: 313*

The bibliography contains 3217 references. This list is representative and care has been taken to include references from every discipline and from every major region of capture and fishery. Only animals of the genus Homaridae are documented.

Available from: Memorial University of Newfoundland,
Marine Sciences Research Laboratory
St. John's, Newfoundland, Canada

Coming Soon!

Bulletin 182

Morphology and Sediments of the Gulf of St. Lawrence

D. H. Loring - D. J. G. Nota

This report describes and assesses the submarine morphology and the sediments (lithology, grain size, mineralogy, and chemistry) of the Gulf of St. Lawrence and the marine estuary of the St. Lawrence River.

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Cheques and money orders should be made payable to the Receiver General of Canada.

Journal of the Fisheries Research Board of Canada

Volume 30, No. 11, November 1973 JFRBAK30(11)1611–1770(1973)

Laboratory studies of the accommodation of some crude and residual fuel oils in sea water
D. C. Gordon Jr, P. D. Keizer, and N. J. Prouse 1611–1618

Factors affecting the behavior of five chlorinated hydrocarbons in two natural waters and their sediments **P. C. Oloffs, L. J. Albright, S. Y. Szeto, and J. Lau** 1619–1623

Fish viruses: isolation and identification of infectious hematopoietic necrosis in eastern North America **K. Wolf, M. C. Quimby, L. L. Pettijohn, and M. L. Landolt** 1625–1627

Beverton-Holt model of a commercial fishery: optimal dynamics
C. Clark, G. Edwards, and M. Friedlaender 1629–1640

Microcultures of brown bullhead (*Ictalurus nebulosus*) cells: their use in quantitation of channel catfish (*Ictalurus punctatus*) **J. B. Gratzek, M. H. McGlamery, D. L. Dawe, and T. Scott** 1641–1645

Digestive enzyme secretion after dietary variation in the American lobster (*Homarus americanus*)
R. J. Hoyle 1647–1653

First records of the gymnoblastic hydroid, *Ichthyocodium sarcotretis*, on the copepod, *Sphyrion lumpi*, from redfish of the northwest Atlantic **W. Templeman** 1655–1660

Fatty acid content of coho salmon, *Oncorhynchus kisutch* — a statistical approach to changes produced by diet **I. J. Tinsley, H. M. Krueger, and J. B. Saddler** 1661–1666

Changes in the population of lake whitefish (*Coregonus clupeaformis*) in Lake Winnipeg from 1944 to 1969 **E. B. Davidoff, R. W. Rybicki, and K. H. Doan** 1667–1682

Divergent growth in two lake whitefish (*Coregonus clupeaformis*) populations **B. F. Bidgood** 1683–1696

Temperatures selected seasonally by four fishes from western Lake Erie
C. A. Barans and R. A. Tubb 1697–1703

Cardiovascular-respiratory activity during recovery from anesthesia and surgery in brook trout (*Salvelinus fontinalis*) and carp (*Cyprinus carpio*)
A. H. Houston, C. L. Czerwinski, and R. J. Woods 1705–1712

Myxosoma cerebralis: isolation and concentration from fish skeletal elements — trypsin digestion method **M. L. Landolt** 1713–1716

Analysis of energy flow and production in an unexploited marine flatfish population
J. C. MacKinnon 1717–1728

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The skate, *Raja richardsoni* Garrick 1961, assigned to *Bathyraja* **W. Templeman** 1729–1732

Occurrence of DDT residues in beluga whales (*Delphinapterus leucas*) from the Mackenzie Delta, N.W.T. **R. F. Addison and P. F. Brodie** 1733–1736

Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus *Poeciliopsis*
M. C. Cimino 1736–1737

Identification of fungal encrustation on the shell of the snow crab (*Chionoecetes bairdi*)
J. M. Van Hyning and A. M. Scarborough 1738–1739

Influence of environmental experience on response of yearling rainbow trout (*Salmo gairdneri*) to a black and white substrate **J. A. Ritter and H. R. MacCrimmon** 1740–1742

Endrin uptake and release by fingerling channel catfish (*Ictalurus punctatus*)
R. L. Argyle, G. C. Williams, and H. K. Dupree 1743–1744

Use of plastein reaction in recovering protein from fish waste **Y. Onoue and V. M. Riddle** 1745–1747

Some aspects of osmoregulation in *Mysis stenolepis* (Crustacea, Mysidacea)
K. A. Dormaar and S. Corey 1747–1749

A portable wire-speed indicator for use with plankton nets
L. D. Lusz and K. D. Waldron 1749–1751

Occurrence of American eels, *Anguilla rostrata*, in waters overlying the eastern North American continental shelf **C. A. Wenner** 1752–1755

An estimate of energy expenditure by rainbow trout (*Salmo gairdneri*) in a small mountain stream
C. R. Feldmeth and T. M. Jenkins Jr 1755–1759

Inability to detect spores of *Clostridium botulinum* in fish protein concentrates (FPC)
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